

COMPARISON BETWEEN A BIOLOGICAL AND A RADIOIMMUNOLOGICAL ASSAY OF PLASMA RENIN CONCENTRATION

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1. Introduction

Variability in incubation conditions for generating angiotensin has made comparison between bioassay and radioimmunoassay of plasma renin activity difficult to interpret. Haas and Goldblatt [1] found that the plasma renin concentration of normal volunteers determined by radioimmunoassay was lower than the bioassay values. Hollemans et al. [2] reported that, with use of the same plasma extracts for assay, the angiotensin concentration was consistently lower by radioimmunoassay than by bioassay. Others have found that values for plasma renin activity obtained by radioimmunoassay are higher than by bioassay [3–5] or comparable to bioassay values [6,7]. We have measured the plasma renin concentration of the same pre-extracted human plasma samples by both a bioassay and a radioimmunoassay technique.

2. Materials and methods

2.1. Extraction of plasma samples

For the renin determination 10 ml blood were collected in an ice cooled tube containing 0.2 ml EDTA (60 mg/ml), centrifuged in the cold and the plasma was frozen at -20°C . The plasma samples were extracted according the method of Skinner [8]. This extraction consists of an irreversible denaturation of the endogenous substrate by dialysis against 0.05 M glycocoll buffer pH 3.3 and of an inactivation of the angiotensinases by heating at 32°C for 1 h.

2.2. Bioassay

The pre-extracted plasma samples were incubated in the presence of an excess sheep substrate (1000 ng/ml) at three appropriate times at 37°C in 0.18 M phosphate buffer pH 7.4. They were assayed for pressor activity against standard (aspartyl¹-valyl⁵)-angiotensine II-amide (Hypertensin, Ciba) in the ganglion-blocked rat.

2.3. Radioimmunoassay

Pre-extracted plasma (0.5 ml), an enzymatic inhibitor mixture of 2,3-dimercaptopropanol and 8-hydroxyquinolinesulphate in water brought to pH 7.4 with 5N NaOH (0.5 ml) and an excess (1 ml) sheep substrate (1000 ng/ml) was pipetted into ice cooled plastic or siliconized glass tubes. After mixing without foaming, 0.5 ml of this mixture was incubated for 1 h in the presence of moist resin (Dowex 50 WX₂ kept as a 15% suspension in 0.18 M phosphate buffer pH 7.4). The generated angiotensin I was eluted with 1 ml 0.1 N diethylamine and 2 ml 0.2 N NH₄ OH [9] and assayed radioimmunologically using a commercial kit supplied by the CEA-IRE-SORIN association. This angiotensin I concentration was corrected by subtracting the value obtained when the assay was carried out at 4°C . Both the bioassay and the radioimmunoassay plasma renin concentrations were expressed as ng angiotensin I generated per ml plasma per hour.

3. Results and discussion

3.1. Recovery of the extraction procedure

The mean recovery, studied by adding known

amounts of standard renin to 4 human plasma samples of a plasma pool with known plasma renin concentration, was $90.3 \pm 6.8(\text{SD})\%$.

3.2. Bioassay

The reproducibility of the bioassay was tested by measuring the plasma renin concentration of a plasma pool at different times in several rat preparations. The mean plasma renin level was $12.85 \pm 0.95(\text{SD})$ ng/ml/h ($n=8$) with a coefficient of variation of 7.4%. The mean difference between two measurements performed at different times in 16 human plasma samples was $10.46 \pm 6.04(\text{SD})\%$.

3.3. Radioimmunoassay

The mean plasma renin concentration determined radioimmunologically on 14 human plasma extracts of a plasma pool was $25.75 \pm 3.48(\text{SD})$ ng/ml/h with a coefficient of variation of 13.5%. The mean percent difference between two determinations on 11 human plasma samples was $10.26 \pm 7.02(\text{SD})\%$. The plasma renin concentration of the same plasma pool was also determined biologically on 6 samples and was $24.67 \pm 4.27(\text{SD})$ ng/ml/h with a variation of 17.3%. There was no significant difference between the plasma renin level determined biologically or radioimmunologically ($p > 0.1$).

3.4. Correlation between methods

The plasma renin concentration of 48 human plasma samples, obtained from patients on dialysis, patients with renal or essential hypertension, was determined in duplicate both by bioassay and radioimmunoassay. 60% of the plasma renin levels determined radioimmunologically was higher than the bioassay values, 28% and 12% respectively lower and comparable. In fig.1 the bioassay values of the plasma renin concentration are plotted against the radioimmunoassay values. A significant ($p < 0.001$) correlation was found between the plasma renin concentration determined biologically (y) and the renin levels obtained radioimmunologically (x): $y = -1.96 + 1.00x$. The high degree of correlation ($r = 0.933$) indicates that the more rapid and convenient radioimmunoassay procedure can be used to measure the plasma renin concentration in human plasma samples instead of bioassay.

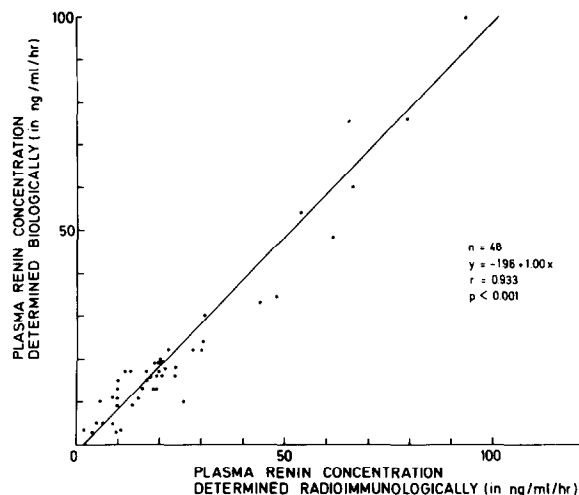


Fig.1. Correlation between bioassay and radioimmunoassay of plasma renin concentration.

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