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ESTIMATION OF 18-HYDROXY-11-DEOXYCORTICOSTERONE (18-OH-DOC) IN HUMAN PLASMA AND URINE INCLUDING A COMPARISON OF VARIOUS TECHNIQUES

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SUMMARY

A comparison between the gas–liquid chromatographic and radioimmunological methods is studied through their respective properties of sensitivity, exactitude and specificity in order to measure the plasmatic and urinary levels of 18-OH-DOC. The high specificity of the antiserum anti 18-OH-DOC-3-carboxy methoxime-BSA is demonstrated. Its application in a non-chromatographic radioimmunoassay in established.

INTRODUCTION

In 1966, the oxidation product of 18-OH-DOC (γ-lactone) was estimated by gas–liquid chromatography in the incubations of rat adrenals [1]. The sensitivity obtained by this method was not sufficient for the measurement of 18-OH-DOC levels in human plasma. Recently, the esterification of the γ-lactone derivative with heptafluorobutyric anhydride (HFB) allowed detection of 100 pg of this steroid [2]. That work reported peripheral levels of 18-OH-DOC in human plasma ranging from 20 to 160 ng per 100 ml with blood being drawn randomly during the day.

Now, the measure of this compound in biological fluids can be performed by radioimmunoassay [3–5, 7, 8]. In this paper, we compare the results obtained by gas–liquid chromatography with electron capture detection with those measured by radiommunoassay. We wish to demonstrate the high specificity of our anti-18-OH-DOC antiserum described elsewhere [3].

MATERIALS AND REAGENTS

All solvents (Merck, Analytical Grade) were distilled before use.

Dioxane was purified on alumina columns before use (Alumina Woelm B—Super I).

Unlabelled 18-OH-DOC was obtained from Searle (Mexico). 18-Hydroxy-11-deoxy-[1,2-^{3}H]-corticosterone (45 Ci/mmol) was purchased from the Radiochemical Center (Amersham), diluted in benzene–ethanol (9:1, v/v) and stored at 4°C.

Helix pomatia gastric juice (Industrie Biologique Française, Gennevilliers) was supplied with a titer of 100,000 U β-glucuronidase (Fishman) and 1,000,000 U sulfatase (Roy).

Plates 20 × 20 cm for thin-layer chromatography were precoated with 0.25 mm of silicagel F 254 (Macheray Nagel).

Heptafluorobutyric (HFB) anhydride was obtained in 1.0 ml pre-scored ampulla (Pierce Chemical Company) and stored at 4°C in a dessicator.

Gas–liquid chromatography (GLC) was carried out using a Packard 7400 instrument equipped with ^{63}Ni electron capture detection. Siliconised pyrex glass columns (1,800 × 4 mm) were packed with gas-chrom Q80/100 mesh (Applied Science Laboratories Inc., PA) coated with a stationary phase of 3% OV17 (Supelco Inc.). The carrier gas (60 ml/min.) was methane–argon (1:9) (Air Product Ltd). Chromatography was carried out at 265°C with detector and flash heater temperatures of 300 and 295°C, respectively.

METHODS

a. Radioimmunoassay

The complete method concerning the determination of 18-OH-DOC in plasma and urine is described in detail elsewhere [3, 7].

b. Gas–liquid chromatography

Plasma. Plasma samples (10 ml) to which 15,000 d.p.m. [^{3}H]-18-OH-DOC had been added, were extracted twice with 30 ml of dichloromethane. The extract was washed consecutively twice with 30 ml of NaOH (0.1 N), then with water and evaporated to dryness. The residue was applied as spots to 20 × 20 cm thin-layer plates and chromatographed in the system benzene–ethyl acetate (1:10, v/v). Standard 18-OH-DOC was chromatographed and located by U.V.-absorption. The corresponding areas of the plasma samples were eluted with ethanol and evaporated. The dried residues were oxidized with periodic

Table 1. The recoveries of [^{3}H]-18-OH-DOC from the γ-lactone-18-OH-DOC HFB region

Conditions	Recovery % (mean ± S.D.)
30 min at 60°C	75 ± 5
60 min at 60°C	75 ± 5
3 h at 58°C	95 ± 2
overnight at room temperature	96 ± 3

acid [6] and extracted three times with 4 ml of methylene chloride. The γ-lactone-18-OH-DOC samples were chromatographed on silicagel in system benzene–acetone (150:50, v/v), located, eluted with benzene and one tenth removed to assess tritiated yields.

The dried remaining eluate was dissolved in 100 μl dry benzene. Heptafluorobutyrate anhydride (100 μl) was then added and the mixture incubated at room temperature overnight. Excess of reagents were removed under a current of N_2 and the residue dissolved in toluene (50 μl). 5–10 μl of this solution were injected into the gas chromatograph.

Urine. The urine was collected during 24 hours. The free and conjugated forms of 18-OH-DOC were studied. For estimation of free 18-OH-DOC, 100 ml urine to which 15,000 d.p.m. [^{3}H]-18-OH-DOC had been added, were extracted three times with 50 ml of dichloromethane. The extracts were washed consecutively with 0.1 N, NaOH and water, and evaporated to dryness. For the conjugated form, 50 ml of urine were adjusted at pH 5.2 by addition of 5 ml acetate buffer 0.1 M (pH 5). The urines were hydrolysed overnight at 37°C by 0.5 ml of *Helix pomatia* gastric juice. The samples were then extracted and treated as described above.

RESULTS

a. Esterification kinetics

Three samples of 20 μg γ-lactone-18-OH-DOC, each of them containing 100,000 d.p.m. [^{3}H]-labelled compound, were esterified in HFB anhydride under various conditions of time and temperature. The HFB derivatives were chromatographed on paper in a cyclohexane–dioxane–methanol–water (40:40:20:10, by vol.) system. Table 1 indicates the recoveries of [^{3}H]-18-OH-DOC from the γ-lactone-18-OH-DOC HFB region under experimental conditions and that esterification is almost quantitative for 3 h at 58°C or overnight at room temperature. Mason and Fraser[2], however, found a quantitative esterification for an incubation at 65°C for 30 min.

Table 2.

Method	*n*	Mean ± S.D. (ng %)
GLC	9	49.7 ± 5.2
RIA	9	54.9 ± 2.7

b. Recovery

The mean recovery of [^{3}H]-18-OH-DOC was 46 ± 9 (S.D.)% after the complete gas–liquid chromatographic procedure. By radioimmunoassay, the recovery of [^{3}H]-18-OH-DOC added to plasma and urine samples was 98 ± 2 (S.D.) %.

c. Linearity of detection response

Electron capture detection response was linear over the range 200–1000 pg.

d. Precision

The precision of each assay was evaluated by multiple measurements of 18-OH-DOC in the same pool of plasma (Table 2).

e. Accuracy

To assess the accuracy of each procedure, known amounts of free 18-OH-DOC were added to urine and plasma. Figure 1 shows the correlation between the "added" and "measured" 18-OH-DOC concen-

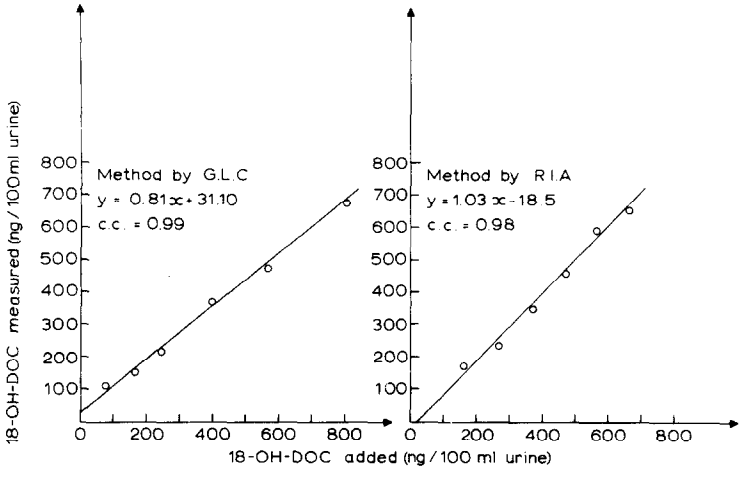

Fig. 1. Recovery of known 18-OH-DOC added to urine by GLC and RIA methods.

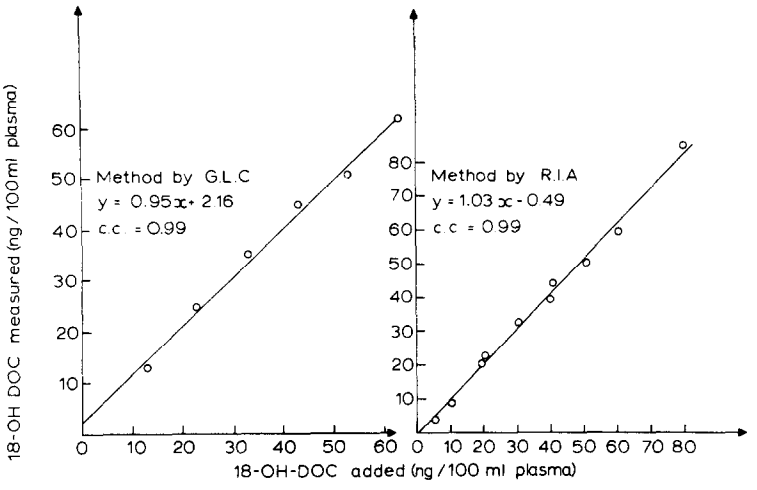

Fig. 2. Recovery of known 18-OH-DOC added to plasma by GLC and RIA methods.

tration, respectively in GLC and RIA, in urine. Figure 2 shows the correlation obtained with plasma samples.

f. Specificity

The plasmatic and urinary HFB-18-OH-DOC lactones demonstrated a single peak with the same retention time as the standard product (Fig. 3). After injection on the chromatographic column, similar results were obtained with a OV1 (3%) or a OV225 (1%) column. Figure 4 represents the standard curve and the specificity of the anti-18-OH-DOC-3-CMO-BSA antiserum, used with a titer of 1:4000 (final dilution). A further study showed a cross-reaction below 1% for 18-OH-corticosterone.

In order to demonstrate the good specificity of our antiserum, plasma ($n = 12$) and urine ($n = 26$) samples were processed on the one hand by radioimmunoassay, on the other hand by the gas–liquid chromatographic method. Figures 5 and 6 show the correlation between the levels of 18-OH-DOC obtained by GLC and RIA, respectively in plasma and urine.

DISCUSSION

18-Hydroxy-11-deoxycorticosterone (18-OH-DOC) levels were estimated in human plasma and urine, by gas–liquid chromatography with electron capture detection and by a radioimmunological method using a specific and sensitive antiserum. The good correlation of plasmatic results obtained by both methods leads us to choose the radioimmunoassay for clinical and experimental use.

As previously described [7], the experimentaly determined values of plasmatic 18-OH-DOC are in good agreement with those reported by other investigators [4, 5] who, recently, developed radioimmunoassay procedures including a chromatographic step. This is an indication of the specificity and the exactitude of our method. Concerning the values of urinary 18-OH-DOC, we found no reference in the literature.

In general, the urinary values of 18-OH-DOC obtained by the GLC method were slightly lower than those measured by radioimmunoassay. Nevertheless, the correlation can be considered satisfactory, having regard to the complexity of the GLC procedure in comparison with radioimmunoassay.

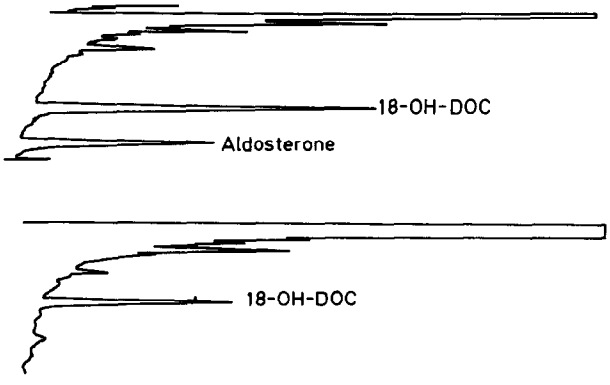

Fig. 3. Gas chromatograms of standard 18-OH-DOC-γ-lactone-HFB and aldolactone-HFB (left) and of a human plasma extract.

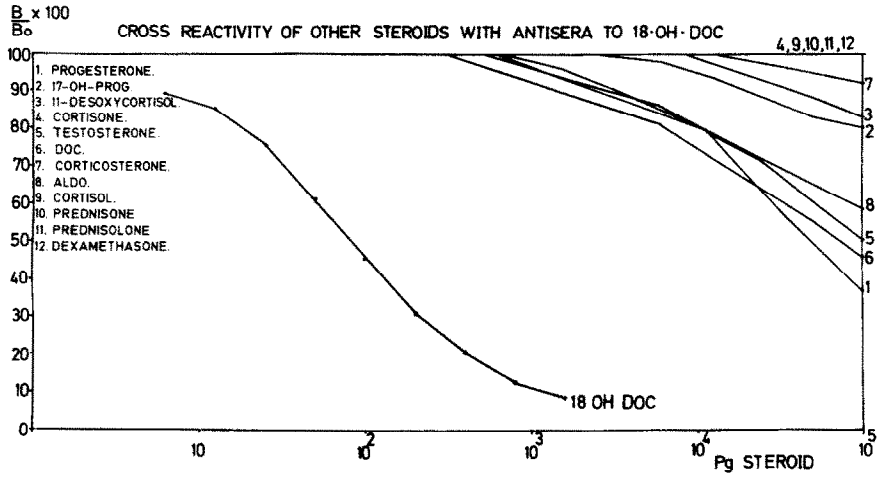

Fig. 4. Displacement curves of [^{3}H]-18-OH-DOC by various steroids in the 18-OH-DOC radioimmunoassay.

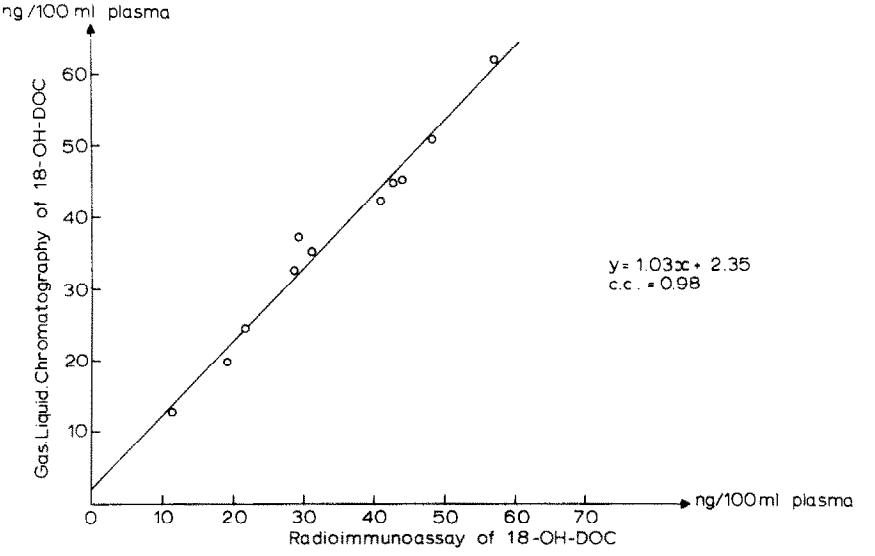

Fig. 5. Comparison of 18-OH-DOC radioimmunoassay with gas–liquid chromatography method in plasma.

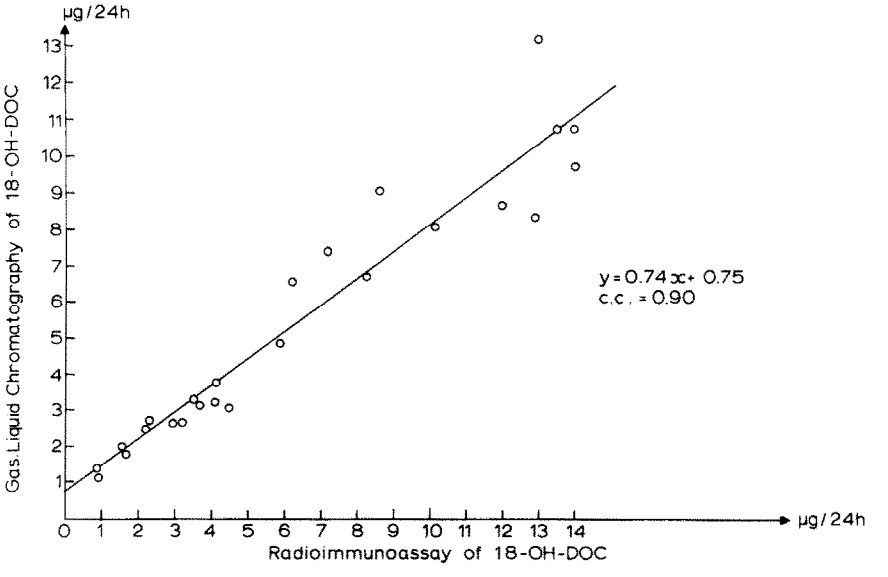

Fig. 6. Comparison of 18-OH-DOC radioimmunoassay with gas–liquid chromatography method in urine.

Besides, we must consider a possible interference of the tetra-18-OH-DOC-derivatives. This latter hypothesis will be studied subsequently.

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