A Non-Chromatographic Radioimmunoassay for Plasma Dexamethasone

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Synopsis

A non-chromatographic radioimmunoassay for plasma dexamethasone is presented. Corticosteroid binding protein (CGB) was used to bind endogenous steroids, and free dexamethasone was adsorbed with Florisil. The purified sample was assayed by using an antiserum to dexamethasone-21-BSA. The plasma blank was less than 10 pg. When 0.1 ml of plasma containing 20 ng of cortisol was measured, the blank could not be differentiated from charcoal treated plasma. ^3H -dexamethasone recovery from purification procedures was $33.2 \pm 4.4\%$. Accuracy and precision were satisfactory. Plasma dexamethasone concentrations in patients receiving dexamethasone administration are also reported.

In radioimmunoassay of plasma dexamethasone, cortisol is a major interfering steroid. In the method reported by Meikle *et al.* (1973), paper chromatography was utilized to eliminate cortisol interference.

Recently, plasma binding proteins have been used for sample purification in radioimmunoassay (Ismail *et al.*, 1972; Martin and Nugent, 1973). On the base that CBG binds endogenous corticosteroids, including cortisol, and a negligible amount of dexamethasone (Morris *et al.*, 1973), an assay using CBG to purify dexamethasone was developed.

Materials and Methods

Materials

Ethyl acetate, n-hexane and methanol (special grade, Wako Pure Chemical Industries) were used without further purification. Florisil (60–100 mesh, Floridin Co.) was washed, and activated at 150°C for 3 hours. $1,2\text{-}^3\text{H}$ -dexamethasone (29 Ci/mMole, The Radiochemical Centre) was purified by thin layer chromatography in the system of chloroform: ethanol

(9: 1). Dexamethasone was obtained from Sionogi and Co. Other materials used were Bovine serum albumin (Wako Pure Chemical Industries), Pepsin-treated human immune serum globulin (Gamma-Venin, Hoechst Japan Co.), Complete Freund adjuvant (Difco Laboratories), $1,2\text{-}^3\text{H}$ -cortisol, $1,2\text{-}^3\text{H}$ -corticosterone, $1,2\text{-}^3\text{H}$ -deoxycortisol, $1,2\text{-}^3\text{H}$ -deoxycorticosterone, $1,2\text{-}^3\text{H}$ -progesterone and $1,2\text{-}^3\text{H}$ -testosterone (New England Nuclear).

Antiserum

Dexamethasone-21-BSA was prepared by following the method of Erlanger *et al.* (1957). Two mg of the conjugate in 0.8 ml saline was mixed with 0.8 ml complete Freund adjuvant, and injected to a rabbit. The injection was given fortnightly for the first 3 months, and monthly thereafter. The antiserum obtained in the 3rd month was useful at 1: 10,000 dilution.

Plasma extraction and purification

One tenth ml of plasma sample and 1 ml of borate buffer (0.05M, pH 7.8) were pipetted into the tube containing 2,000 cpm of ^3H -dexamethasone. The solution was incubated in ice water bath for 30 min. Eighty mg of Florisil was added to the sample, and shaken for 1 min. After the supernatant was removed, the Florisil was washed twice with 1 ml of borate buffer. The Florisil was extracted in a mixture of 1 ml of water with 2 ml of ethyl acetate: n-hexane (7: 3). The ethyl acetate: n-hexane layer was aspirated to another tube, and

the water layer was discarded. One ml of water was pipetted to the Florisil, and it was extracted with 2 ml of ethyl acetate. The ethyl acetate was aspirated to the tube containing ethyl acetate: n-hexane, and evaporated. Then, after adding 0.1 ml of charcoal treated plasma to the residue, the procedures of Florisil adsorption and extraction were repeated. The residue was redissolved in 1 ml of methanol. Two tenth ml aliquot was counted for recovery.

Radioimmunoassay

For radioimmunoassay, 0.1 to 0.2 ml of plasma extracts dissolved in methanol and standards containing 10, 20, 30, 50, 100 and 200 pg of dexamethasone were pipetted into assay tubes, and dried. Then, 0.25 ml of appropriately diluted antiserum with 3,000 cpm ^3H -dexamethasone in borate buffer containing 0.1% pepsin treated human immune serum globulin and 0.06% BSA was added to the sample. After incubation overnight at 4°C, 0.25 ml of saturated ammonium sulfate was added, the mixture was stirred, and centrifuged at 3,000 rpm for 15 min. The radioactivity in 0.25 ml of the supernatant was counted in the Aloka model LSC-502 liquid scintillation counter.

Plasma cortisol

Plasma cortisol was estimated by the radioassay method of Murphy (1967).

Results

Standard curve

A typical standard curve in the range of 0 to 200 pg dexamethasone is shown in Figure 1.

Blank

Blank of charcoal treated plasma was less than 10 pg. When plasma containing less than 20 ng of cortisol was measured, the blank could not be differentiated from steroid-free plasma (Table 1).

Recovery, specificity and sensitivity

The rate of recovery of dexamethasone from the purification procedures was $33.2 \pm 4.4\%$. (Table 2).

Cross reactions of various endogenous steroids were determined at the 1 ng level for dexamethasone antiserum. The interference by endogenous steroids could be reduced after

Fig. 1. Standard curve for dexamethasone.
(mean $\pm$ SD, n = 5)

Table 1. Cortisol interference

Cortisol added (ng)	Dexamethasone added (pg)	Dexamethasone measured (pg)
0	0	undetectable
10	0	undetectable
20	0	undetectable
30	0	125*
40	0	375*
50	0	440*
0	300	289
10	300	306
20	300	340
30	300	481

Steroids were added in 0.1 ml of charcoal treated plasma.

*Values were expressed as dexamethasone equivalent.

CBG purification. When 300 pg of dexamethasone was added to plasma containing 0, 10, 20 and 30 ng of cortisol, the recovery was 289, 306, 340 and 481 pg, respectively. (Table 1).

Table 2. Steroids recovery from purification, antibody cross reactivity and overall specificity of the assay

	Recovery (%)	Cross reactivity* (%)	Overall specificity† (%)
Dexamethasone	33.2	100	100
Cortisol	1.7	3.5	0.18
Corticosterone	1.2	3.9	0.14
Deoxycortisol	1.8	3.6	0.20
Deoxycorticosterone	2.0	9.1	0.55
Progesterone	1.7	3.7	0.19
Testosterone	5.5	10.9	1.8
Betamethasone		7.9	
Prednisolone		6.7	

* Values were based on the competition of 1 ng of each steroid with ^3H -dexamethasone for binding sites on the antibody.

† For example: cortisol = $1.7/33.2 \times 3.5$

The minimal detectable amount of dexamethasone on the standard curve was 8 pg.

Precision

In order to determine intra-assay precision, 200 and 500 pg of dexamethasone were added to 0.1 ml of steroid-free plasma. The coefficient of variation was 13.8% and 9%, respectively ($N = 6$). When these samples were analyzed on different days, the coefficient of variation were 12.8% and 14.6%, respectively.

Table 3. Plasma dexamethasone

Plasma cortisol ($\mu\text{g/dl}$)	Plasma dexamethasone (ng/dl)	Dexamethasone administered (mg)	Time after administration (hrs)
2.7	undetectable	0	—
7.5	undetectable	0	—
15.3	undetectable	0	—
20.8	undetectable	0	—
*1.9	301	0.5	5
1.7	238	1	7
21.5	undetectable	1	10
7.6	undetectable	1	10
7.8	623	8	10
9.3	768	16	10

* Plasmas were from patients receiving oral dexamethasone treatment or suppression test.

Accuracy

Accuracy was examined by adding 200, 500, 1000 pg of dexamethasone to 0.1 ml of charcoal treated plasma, and analyzing them. The recovery was 111.7 ± 15.5 , 97.4 ± 8.8 and $99.3 \pm 10.4\%$, respectively.

Plasma concentration

Plasma dexamethasone levels after receiving oral dexamethasone administration are shown in Table 3 and Figure 2. When plasma dexamethasone and cortisol were estimated after 1 mg oral dose of dexamethasone

Fig. 2. Plasma dexamethasone and cortisol levels after a single dose of dexamethasone (1 mg).

thasone, dexamethasone levels reached a peak of 521 ng/dl in 1 hr, and decreased to 238 ng/dl in 7 hr. Plasma cortisol level decreased from 9.3 μ g/dl to 1.7 μ g/dl in 7 hrs, and returned to 4.2 μ g/dl in 24 hrs while plasma dexamethasone was undetectable.

Discussion

A non-chromatographic radioimmunoassay for plasma dexamethasone is reported. The endogenous corticosteroids bound to CBG were separated from free dexamethasone which was adsorbed with Florisil. Although the recovery of 3 H-dexamethasone in present method was lower than those in radioimmunoassays containing chromatographic purification, accuracy and precision were satisfactory. After CBG purification, recovery of most of the endogenous steroids were about 2% or less, with the exception of testosterone (Table 2). In plasma obtained from normal person, endogenous steroids in this concentration will not significantly interfere with the assay. However, in patients with hypercorticism or pregnant women, the high level of cortisol and progesterone will give rise to significant interference. The plasma values determined after oral dexamethasone administration approximate those which have been reported (Meikle *et al.*, 1973) and the values were depended on subject, dosage and time of sampling. The method presented here

is less time consuming by eliminating chromatography and is expected to be a simple method suitable for routine use in estimating plasma dexamethasone in patients receiving dexamethasone administration, almost all of them plasma cortisol is suppressed.

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