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MEASUREMENT OF PLASMA PREDNISOLONE IN MAN

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1 A modification of the competitive protein binding assay for prednisolone described by English, Chakraborty & Marks (1974) is presented.

2 Addition of pg quantities of [^{3}H]-prednisolone marker to each sample allows a more reliable estimation of plasma prednisolone following oral dosage with prednisone or prednisolone.

3 Mean plasma half-life and peak plasma concentrations in patients with respiratory disease seen following prednisolone administration agree well with previous studies in healthy volunteers performed by other workers using competitive protein binding and radioimmunoassay techniques.

4 A considerable inter-patient variation in bioavailability after a standard dose of prednisolone (20 mg) was noted.

Introduction

Corticosteroid therapy is part of the management of a variety of clinical disorders (Bevan, Espiner & Donald, 1970). Prednisolone and prednisone, synthetic corticosteroid analogues, are now the most commonly used oral agents due to their potent glucocorticoid properties (Beaven *et al.*, 1970; Sandberg, Bacallao & Cleveland, 1970), and although these drugs have been widely used for over ten years, little information is available relating the pharmacokinetic parameters of these drugs to their therapeutic effect.

Recently, several groups have developed sensitive methods for the estimation of plasma prednisolone based on competitive protein binding or radioimmunoassay (English *et al.*, 1974; Turner, Carroll, Pinkus, Charles & Chattoraj, 1973; Colburn & Buller, 1973).

In the present study, existing methods were modified to achieve greater accuracy in the determination of prednisolone plasma levels in patients treated with prednisolone or prednisone.

Methods

Steroids

1, 2- [^{3}H]-cortisol and 6, 7- [^{3}H]-prednisolone (specific activities 45 Ci/mmol and 47 Ci/mmol respectively) were obtained from the Radiochemical Centre, Amersham, Bucks. [^{3}H]-cortisol was dissolved in saline containing 2% ethanol to give a working solution of 1 μCi/ml. [^{3}H]-prednisolone was similarly dissolved to give a working solution of 0.1 μCi/ml. Radiochemical purity was checked by thin-layer chromatography on Kodak Chromatogram sheets (silica gel type 13181), using dichloromethane/methanol/water (150:10:1) as developer. Cortisol and prednisolone were purchased from Sigma Chemical Company.

Binding protein-isotope reagent

Binding reagent was prepared immediately before use by the addition of 1 ml of tritiated cortisol (1 μCi/ml) to distilled water (20 ml) in a 25 ml volumetric flask, adding plasma (1 ml) and making up the volume to 25 ml (Pegg & Keane, 1969).

Dextran-coated charcoal suspension

This was prepared by mixing equal volumes of a 1% aqueous suspension of charcoal (Norit A, Sigma Chemical Company) and a 0.1% aqueous solution of Dextran T70 (Pharmacia Ltd, Uppsala, Sweden).

Isotope counting

Radioactivity was counted using a Packard Tricarb Model 3375 liquid scintillation spectrometer. Packard 'Instagel' was used as scintillant. Counting efficiency was determined using automatic external standardization.

Sample extraction and purification

Plasma samples (2 ml) were extracted with dichloromethane (12 ml) after addition of 20 µl of [^{3}H]-prednisolone solution (2nCi) and sodium chloride (1 g) in duplicate. The samples were centrifuged at 3000 g for 10 min at 5°C in a Mistral 2L centrifuge. The upper aqueous layer was then aspirated off and the dichloromethane separated from residual water and denatured protein by filtration through Whatman 1 PS phase-separating paper. Each extract was evaporated to dryness in 50 ml wide-necked tubes at 40°C under dry nitrogen and the residue taken up in enthanol (100 µl).

An aliquot (60 µl) from each sample was applied as a streak 2 cm long at the bottom of a previously activated silica gel sheet (70°C, 30 min) containing a fluorescent indicator (Kodak Chromagram Type 13181). Prednisolone and cortisol markers (10 µg) were applied alongside the samples and the chromatogram developed in dichloromethane/methanol/water (150:10:1) until the solvent front had travelled 15 cm.

The markers were located under u.v. light (254 nm) and the sample areas alongside the prednisolone marker cut out (avoiding the cortisol marker) and placed in acetone (5 ml). After overnight elution, each sheet was removed, rinsed with acetone and the rinsings pooled with the respective sample eluate. The eluates were evaporated to dryness and the residue dissolved in saline containing 2% ethanol (2 ml). Aliquots (1 ml) of this solution were used for the assay of prednisolone by competitive protein binding as described for cortisol by Pegg & Kearne (1969). Samples found to have prednisolone concentrations outside the useful range of the curve (<50 ng/ml) were diluted in saline/ethanol and the estimations repeated.

Aliquots of undiluted sample (0.2 ml) were counted to calculate recovery of prednisolone during the purification procedure. Prednisolone standards (75 and 150 ng/ml) in prednisolone-free plasma and blank samples were included in the assay.

Competitive protein binding

Prednisolone standards (0-100 ng in 1 ml 2% ethanol/saline) and plasma extracts (1 ml) were pipetted into labelled centrifuge tubes. Plasma-isotope solution (1 ml) was added to each sample and the samples mixed on a vortex mixer. The samples were placed in a water bath at 45°C (5 min) and then transferred to a water bath at 4°C for 10 minutes. Dextran-coated charcoal solution (0.5 ml), kept continually stirring at 4°C, was added, the samples vortex-mixed and then centrifuged at 3000 *g* at 5°C for 5 minutes. Aliquots (1 ml) of the supernatant were transferred to vials containing scintillant (9 ml), and counted. To determine the total radioactivity 1 ml of the plasma isotope solution was added to 2% ethanol/saline (1.5 ml), mixed and 1 ml counted. The standard curve was then plotted as % ^{3}H bound

$$\frac{\text{Supernatant d/min/ml}}{\text{Added d/min/ml}} \times \frac{100}{1}$$

versus prednisolone concentration. The prednisolone in the unknown samples was read from the standard curve (Figure 1), the blank values subtracted and then the samples corrected for dilution and recovery.

Patients

Patients in the study groups were hospitalized for the treatment of various respiratory illnesses and were receiving prednisolone or prednisone as part of their treatment. Two studies were carried out. In the first study, blood samples were taken 2 h after ingestion of prednisolone or prednisone at dosages between 2.5 and 25 mg. In the second study, eight patients received a standard dose of prednisolone (20 mg) and blood samples taken at intervals between 1 and 8 h in order to obtain more detailed plasma profiles. All other drugs were continued during these studies.

Results

Sensitivity and precision of assay

The lowest concentration of prednisolone that can be measured with confidence (duplicates differing by less than 10%) using this technique was found to be 20 ng/ml.

Twenty-two samples in which prednisolone had been added to prednisolone-free plasma at a concentration of 75 and 150 ng/ml were analysed and the mean prednisolone concentrations (± coefficient of variation) found to be 75.2 ± 10.37% (n = 10) and 151.0 ± 8.6% (n = 10) ng/ml respectively. Samples in which duplicate values varied appreciably (more than 15%) were repeated. The average recovery of [^{3}H]-prednisolone added as a marker was 42.5 ±7% (n = 18). When corrected for dilution this figure was found to be 70.8 ± 11.7%, which is comparable with the figures obtained by English *et al.* (1974), and Turner *et al.* (1973).

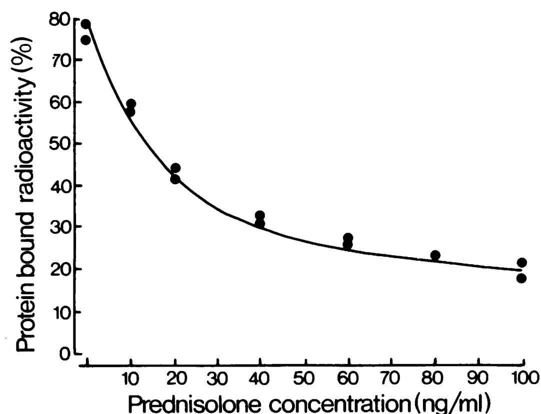

Figure 1 Competitive protein binding curve for prednisolone.

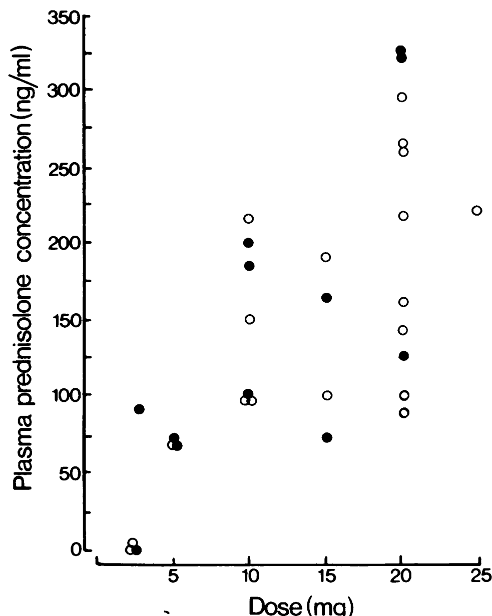

Figure 2 Plasma prednisolone concentration 2 h after ingestion of prednisolone (○) or prednisone (●).

Plasma levels achieved at 2 hours

Following oral dosage with prednisolone or prednisone, blood samples were taken at 2 h after ingestion in 30 patients on chronic steroid therapy at dosages varying from 2.5 to 25 mg. The results of these studies are shown in graphical form in Figure 2. It was noted that plasma prednisolone concentration increased with dose, but a large variation in plasma level was achieved in different patients. The values obtained are however in agreement with the data of other groups (Table 1). There was no apparent difference in the 2 h prednisolone level between those patients who received prednisone as opposed to prednisolone.

The conversion of prednisone to prednisolone is rapid and other workers have found that the plasma profiles achieved in the same patient following prednisone or prednisolone administration were similar (Jenkins & Sampson, 1967).

Prednisolone half-lives and profiles

The plasma prednisolone profiles of the eight patients receiving prednisolone (20 mg) are shown in Figure 3.

Plasma prednisolone half-lives were obtained for each profile by constructing a log-linear regression by the method of least squares, using a

Table 1 Pharmacokinetic parameters associated with prednisolone and prednisone

Author	*Steroid*	*Dose (mg)*	*Plasma prednisolone concentration 2 h after dose (ng/ml per mg dose)*	*Status of subject*	$T_{\frac{1}{2}}$ *(h)*
Sandberg *et al.* (1970)	Prednisolone	20	13	Ten normal males	2.35
D'Arcy *et al.* (1971)	Prednisolone	90	11	Six normal males	2.7
English *et al.* (1974)	Prednisolone	15	8.4	Six normal males	2.5
Colburn & Buller (1973)	Prednisone	60	12	Four normal males	3.0
Turner *et al.* (1973)	Prednisone	10	5	Ten normal males	—
Sullivan *et al.* (1974)	Prednisolone	10	20.9	Twelve normal males	2.9
Present study	Prednisolone	20	11	Eight patients with respiratory disease	2.2

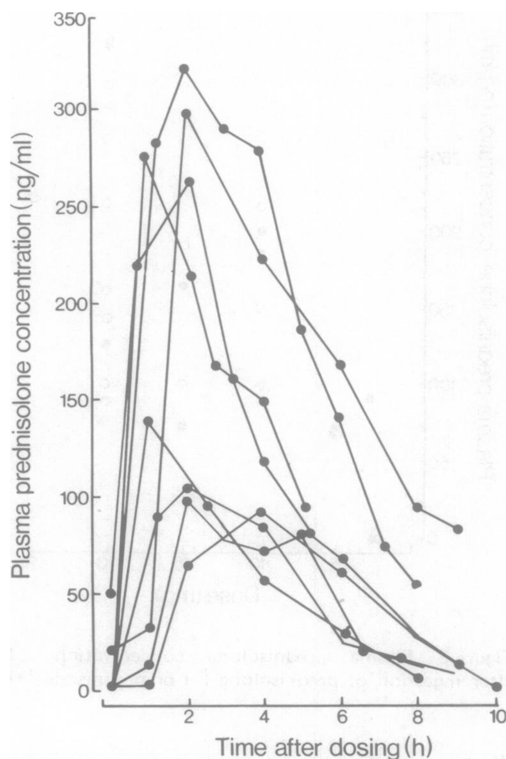

Figure 3 Plasma prednisolone profiles in eight patients after receiving prednisolone (20 mg).

Hewlett Packard programmable calculator and an X, Y plotter. Mean plasma half-life was 2.16 ± 0.74 hours.

Discussion

Modification of the competitive protein binding method of prednisolone assay as described by English *et al.* (1974) has facilitated the measurement of plasma prednisolone in patients receiving steroid therapy. It is principally during chromatography that loss of prednisolone occurs since extraction with dichloromethane recovered 90-92% of the added radioactivity. The addition of [^{3}H]-prednisolone as a marker to each sample allows calculation of prednisolone lost during this step. The amount of prednisolone marker added (less than 1 pg) is much less than the minimum detectable level of prednisolone in the plasma sample (20 ng/ml) and the radioactivity due to the marker ^{3}H in the prednisolone sample after purification (500 → 1000 d/min/ml) is much less than the [^{3}H]-cortisol (80,000 d/min/ml) added to each sample during the competitive binding step. The addition of [^{3}H]-prednisolone therefore allows accurate calculation of recovery without interfering with the competitive protein binding assay.

The use of dichloromethane rather than ethyl acetate as an extraction solvent has been recommended (Cope, 1964) since dichloromethane does not extract polar drug metabolites. Dichloromethane is however difficult to isolate after extraction since it forms a lower phase. This problem has been overcome by the use of phase-separating paper. Radioimmunoassay techniques may be applied to the estimation of plasma prednisolone (Colburn & Buller, 1973; Sullivan, Stoll, Sakmar, Blair & Wagner, 1974). Cortisol however cross-reacts with the primary antibody and therefore interferes in this technique. Even though oral prednisolone rapidly depresses cortisol secretion to basal levels of <5 μg/100 ml (D'Arcy, Griffin, Jenkins, Kirk & Peacock, 1971; Shenfield, Paterson, Costello & Ijadvola, 1974), the interference will be considerable at lower prednisolone levels using this technique. The thin-layer chromatographic step in our method avoids this interference and also separates prednisolone from other drugs or drug metabolites which might otherwise interfere.

The need to monitor plasma levels of drugs in order to design a drug dosage schedule has been advocated by many workers (Koch-Weser, 1972; Vessel & Passananti, 1971). Koch-Weser (1972) has stated that 'It is not generally appreciated that the variability of the dose-effect relationship among patients is primarily due to individual differences in the serum concentration achieved with a given dose schedule rather than to a different intensity of action associated with the same serum concentration.' This situation may well apply to the therapeutic effects of prednisolone and more especially to side effects associated with this drug.

We have attempted to demonstrate a relationship between peak plasma prednisolone concentration and dose of drug administered. From a study of our profiles (see Figure 3) and from examination of other workers' data, 1-2 h was most commonly the interval at which peak plasma levels occurred after oral administration. We therefore selected 2 h after oral administration as the test time.

The large variation in plasma prednisolone levels seen both in the 2 h and the profile studies suggest that a number of factors may influence the bioavailability of the drug. Kozower, Veatch & Kaplan (1974) have mentioned some of the parameters influencing plasma levels e.g., body size, binding to tissues and to serum proteins with

altered distribution in body compartments and rates of drug elimination. The variation in the plasma level achieved after oral dosage, in our studies appear to be due to differences in both absorption and elimination rate.

Mean prednisolone half-life (± s.e. mean) was found to be 2.16 ± 0.66 h in the eight patients studied, a figure in approximate agreement with the results of other workers (Table 1), although the majority of other studies have been carried out with healthy volunteers and few groups have studied pharmacokinetic parameters associated with patients on chronic steroid therapy. Kozower *et al.* (1974) measured the disappearance of plasma radioactivity following the intravenous administration of [^{3}H]-prednisolone, and found that patients who show side effects have longer plasma half-lives for prednisolone (measured as ^{3}H) than those subjects who show no side effects. Blood levels of prednisolone were not measured in their study. This group was of the opinion that variation in the rate of drug elimination is the most important factor in deciding whether or not side effects develop.

We have studied the plasma prednisolone profile in patients receiving a standard dose of prednisolone (20 mg) and eight of the profiles are presented here (Table 1 and Figure 3). Peak plasma levels of the steroid, following oral dosage, occurs at 1-3 hours. The 2 h plasma level was representative of the plasma level obtained by the patient. We have noted a large intersubject variation in the peak prednisolone level and the area under the plasma concentration varies in our studies and those of Sandberg *et al.* (1970) and Sullivan *et al.* (1974). Moreover, the difference is evident even in patients of similar weight and elimination half-life.

Our studies suggest therefore that variations in absorption of prednisolone occur and we propose that absorptive differences might be as important as variation in elimination rate in determining the peak plasma levels and the area concentration curves.

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