Br. J. clin. Pharmac. (1975) 2, 327-332

PREDNISOLONE LEVELS IN THE PLASMA AND URINE: A STUDY OF TWO PREPARATIONS IN MAN

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1 A competitive protein binding method was used to measure prednisolone and cortisol in the blood and urine of volunteers given prednisolone by mouth.

2 Each subject received a single small dose of prednisolone (15 mg) in the standard tablet form and a fortnight later a regulated release formulation of prednisolone metasulphobenzoate containing an equivalent amount of prednisolone.

3 Plasma prednisolone levels rose rapidly after the standard tablet and more slowly after the regulated release form. The normal activity of the hypothalamic-pituitary-adrenal axis as measured by the 09.00 h plasma cortisol concentration was present 24 h after ingestion of the regulated release preparation. In contrast, the 09.00 h plasma cortisol level was reduced in subjects 24 h after receiving prednisolone in the standard tablet form.

Introduction

Although a wide range of synthetic corticosteroids is now available, prednisolone (Δ^1-cortisol) still remains the most popular choice in glucocorticoid therapy, especially for oral administration. Standard preparations of prednisolone alcohol given orally are rapidly absorbed within 1-2 h and the reported plasma half-life after intravenous administration in the human is 192-220 min (Ely, Done & Kelley, 1956; Sandberg & Slaunwhite, 1957; D'Arcy, Griffin, Jenkins, Kirk & Peacock, 1971; Colburn & Buller, 1973; Turner, Carroll, Pinkus, Charles & Chattoraj, 1973; English, Chakraborty & Marks, 1974).

In order to achieve better therapeutic efficacy and least side-effects, repeated dosage of small amounts of the drug in standard tablet form or the use of sustained release formulations has been recommended (Wagner, Carpenter & Collins, 1960; Harter, Reddy & Thorn, 1963; Grant, Forsham & di Raimondo, 1965; Ackerman & Nolan, 1968; D'Arcy *et al.*, 1971). Because of the paucity of information on the plasma concentration after small doses of prednisolone in the clinical situation, the size of dose used and its frequency remains arbitrary. Now that sensitive methods for measuring prednisolone are available (Sandberg, Bacallao & Cleveland, 1970; Colburn & Buller, 1973; Turner *et al.*, 1973; English *et al.*, 1974), it should be possible to relate plasma prednisolone levels to dosage regimes. Such investigations are likely to be useful in the management of patients on long term treatment and may provide a suitable model for the assessment of any new formulation of prednisolone.

We report here a study on plasma and urinary prednisolone and cortisol in healthy male volunteers after a single small dose of two preparations of prednisolone.

Methods

Materials

[1,2-^{3}H] cortisol (45 Ci/mmol) and [6,7-^{3}H] prednisolone (47 Ci/mmol) were obtained from the Radiochemical Centre, Amersham, England. They were dissolved in ethanol to give a working solution of 10 μCi/ml and stored at 4°C. Cortisol and prednisolone were purchased from Sigma Chemical Company, London. In the human studies standard prednisolone tablets (5 mg) were obtained from Rousell Laboratories, Middlesex. Prednisolone metasulphobenzoate was prepared in a regulated release form by Pharmax Limited, Kent.

Thin layer chromatography was carried out on silica gel sheets containing a fluorescent indicator (Chromogram 13181, Eastman-Kodak). All other reagents were of standard laboratory grade.

Plasma prednisolone and cortisol assay

A competitive protein binding (CPB) method was used for the simultaneous measurement of prednisolone and cortisol in the plasma samples (English *et al.*, 1974).

Prednisolone and cortisol measurement in urine

The method previously used for plasma (English *et al.*, 1974) was applied to urine. A 1 ml aliquot of urine was extracted with ethyl acetate (5 ml x 2). The extracts were combined, evaporated to dryness and chromatographed on silica gel sheets in the solvent system methylene dichloride/methanol/water (150:10:1). The prednisolone and cortisol bands were eluted with acetone (2 ml x 2) and the CPB assay carried out as described for plasma.

Isotope counting

Radioactivity was determined using a Packard Liquid Scintillation Counter, Model 2425, fitted with an external standard for monitoring the efficiency of counting. The scintillation fluid was toluene-Triton X 100 (2:1) containing 0.6% butyl PBD [2-(4^{1}-tert-butylphenyl)-5-(4^{11}-biphenylyl)-1,3,4-oxadiazole].

Human studies

Six healthy male volunteers aged between 25-35 years participated in the study. All studies were made on overnight fasting subjects. Three experiments were carried out at fortnightly intervals. In experiment 1 the normal diurnal changes in plasma cortisol were observed during the experimental period used subsequently, i.e., 09.00 h-21.00 hours. In experiments 2 and 3 each subject fasted overnight and at 09.00 h received prednisolone (15 mg) by mouth, either in the form of prednisolone alcohol (3 x 5 mg tablets) or prednisolone metasulphobenzoate (Pharmax) made up as a single regulated release tablet.

Venous blood was collected before and at 1, 3, 5, 7 and 24 h after prednisolone ingestion. Plasma was separated immediately and stored frozen until assayed. Twenty-four hour urine samples were collected and stored at 4°C.

Prednisolone and cortisol were measured as previously described.

Determination of dissolution rates

The dissolution rate *in vitro* of each preparation containing prednisolone (30 mg) was evaluated by the standard rotating basket method (United States Pharmacopoeia XVIII, 1970). The agitation rate used was 500 rev/minute.

Results

Urine assay

The results given in Table 1 show that the CPB method previously used to measure prednisolone in plasma can also be applied to urine. Accuracy was assessed by calculating the recovery of known amounts of prednisolone added to the urine at three different concentrations (1, 2 and 3). The recoveries were within the 83-85% range. Replicate analyses of these and other urine samples permitted estimation of the precision of the assay (Table 1).

Human studies

Plasma prednisolone levels in individual subjects at various time intervals after ingestion of a single oral dose of 15 mg prednisolone alcohol are shown in Figure 1. They illustrate, in agreement with other workers (Jenkins & Sampson, 1967; D'Arcy *et al.*, 1971; Colburn & Buller, 1973; Turner *et al.*, 1973) that prednisolone is rapidly absorbed and peak plasma prednisolone concentrations are achieved within the first hour after dosing. The average peak plasma concentration was 139 ± 7 ng/ml and the average plasma half-life was

Table 1 Accuracy and precision of prednisolone assay in urine

Sample*	Amount of prednisolone added/assay tube (ng)	Prednisolone values determined by the method (Mean + s.d.)	Coefficient of variation	% Recovery
1	5	4.24 ± 0.05	4%	85
2	25	21.28 ± 0.13	2%	85
3	50	42.56 ± 0.24	2%	85

* In each case, ten replicate analyses were made of aliquots of urine (2 ml) to which prednisolone had been added.

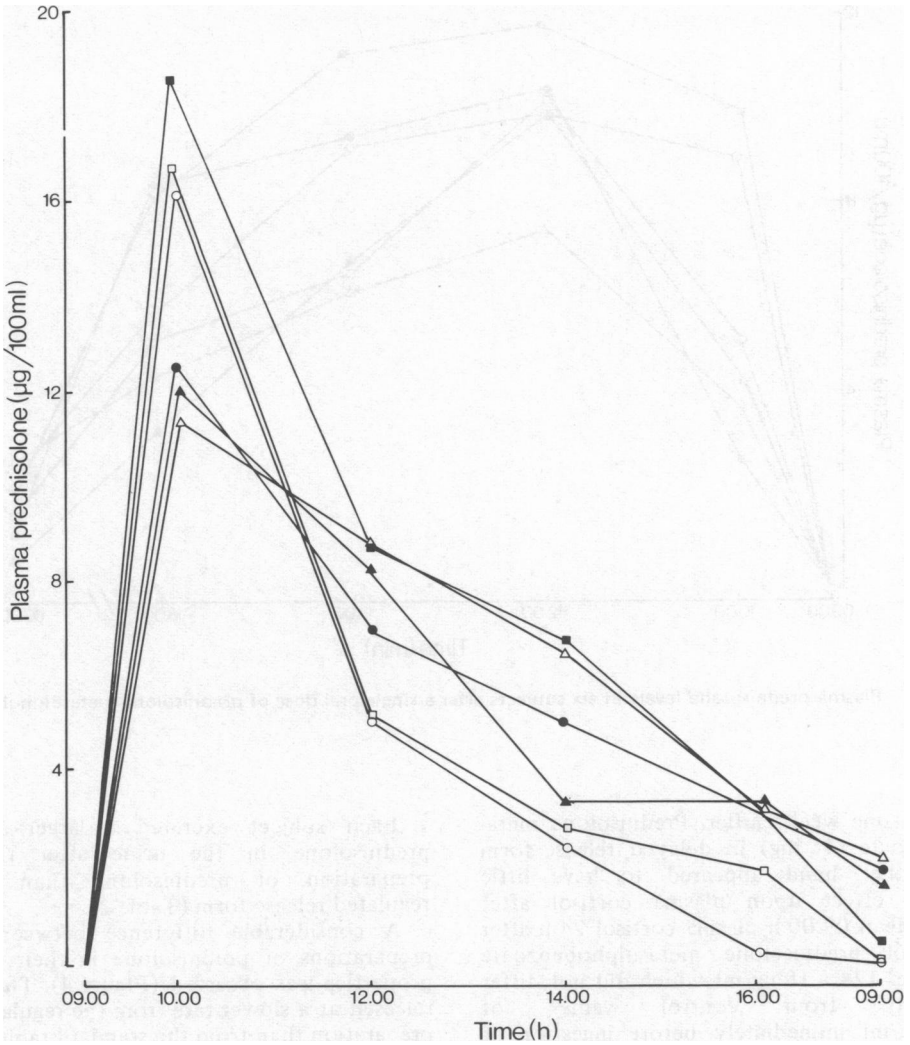

Figure 1 Plasma prednisolone levels in six subjects after a single oral dose of prednisolone alcohol (15 mg).

3.5 ± 0.34 hours. In three out of the group of six subjects a small amount (<20 ng/ml) of prednisolone was detectable in the plasma 24 h after ingestion (Figure 1).

A different plasma prednisolone pattern was observed after ingestion of a regulated release preparation of prednisolone metasulphobenzoate (Figure 2). Plasma prednisolone levels rose more slowly and reached their peak after 3 hours. The mean peak value (100 ± 16 ng/ml) was significantly ($P< 0.01$) smaller than after ingestion of the same amount of prenisolone in regular form (139 ± 7 ng/ml). After 24 h the amount of prednisolone remaining in the plasma was indistinguishable between the two different preparations.

Suppression of plasma cortisol by prednisolone, though present, was not very great during the first few hours, i.e., 09.00 h-17.00 h of the experimental period, but was much more marked the following morning (Figure 3). The mean 09.00 h plasma cortisol in the six volunteers 24 h after they had taken prednisolone (15 mg) in regular tablet form was 79 ± 7 ng/ml compared with a mean value of 143 ± 9 ng/ml in the same group of subjects immediately preceding ingestion of the prednisolone. This latter value does not differ significantly from that obtained during the control

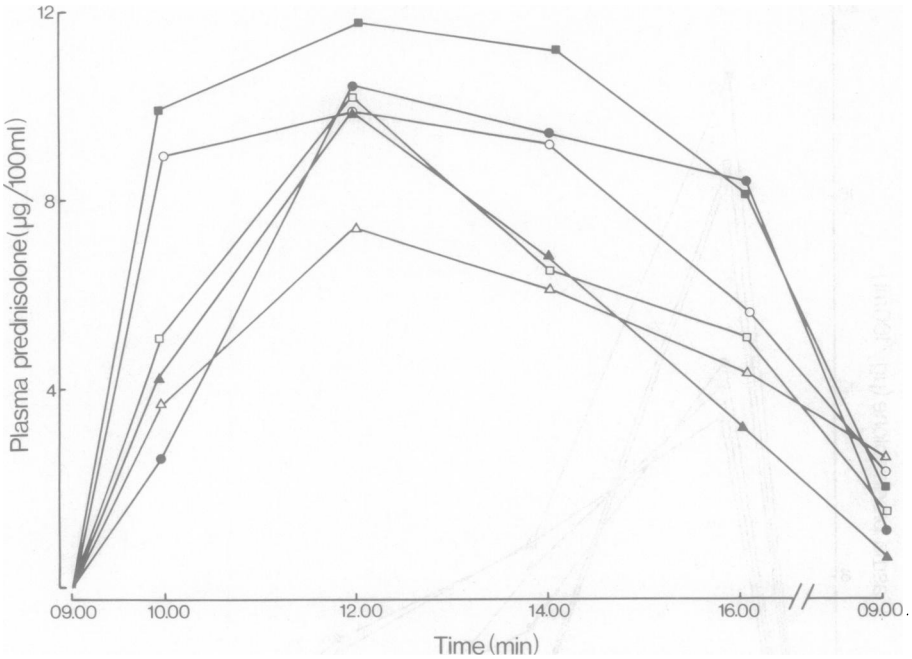

Figure 2 Plasma prednisolone levels in six subjects after a single oral dose of prednisolone metasulphobenzoate (15 mg).

experiment one week earlier. Prednisolone metasulphobenzoate (15 mg) in delayed release form on the other hand appeared to have little suppressive effect upon plasma cortisol after 24 hours. Mean 09.00 h plasma cortisol 24 h after ingestion of prednisolone metasulphobenzoate (15 mg) was 124 ± 18 ng/ml which did not differ significantly from control values of 157 ± 16 ng/ml immediately before ingestion of tablets.

Each subject excreted a larger amount of prednisolone in the urine after the regular preparation of prednisolone than after the regulated release form (Table 2).

A considerable difference between the two preparations of prednisolone in their dissolution properties was observed (Figure 4). The drug was released at a slower rate from the regulated release preparation than from the standard tablet.

Table 2 Excretion of prednisolone in the 24 h urine after oral administration of prednisolone alcohol and prednisolone metasulphobenzoate.

	Amount of prednisolone (µg) excreted/24 h after:			
	Prednisolone alcohol		*Prednisolone metasulphobenzoate*	
Subject	*µg/24 h*	*% dose/24 h*	*µg/24 h*	*% dose/24 h*
1	1536	10.2	104	0.69
2	1458	9.7	139	0.93
3	1474	9.8	99	0.66
4	1674	11.2	52	0.35
5	1142	7.6	35	0.23
6	2332	15.6	63	0.42

Urine aliquots (2 ml) were analysed for prednisolone by the method described in the text.

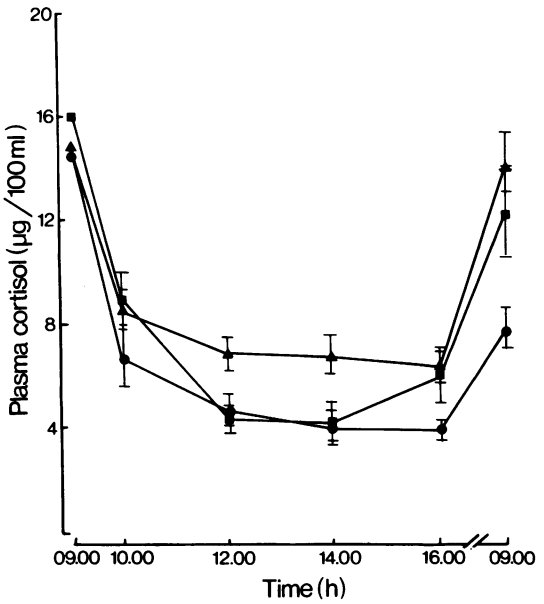

Figure 3 Plasma cortisol levels showing the normal diurnal rhythm (▲) and the effect of a single oral dose of prednisolone alcohol (15 mg, ●) or prednisolone metasulphobenzoate (15 mg, ■). The results shown are the mean ± s.e. mean for six subjects.

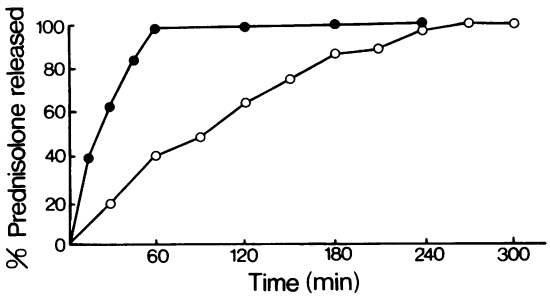

Figure 4 The dissolution rates of two preparations of prednisolone, a standard tablet form (●) and a regulated release preparation (○). Tablets containing prednisolone (30 mg) were used for each time point. Each determination was performed in duplicate.

Discussion

The strikingly different pattern in plasma prednisolone produced by the two prednisolone formulations is consistent with their respective dissolution properties *in vitro*. It would also appear to be responsible for the marked differences in plasma cortisol response and urinary prednisolone excretion produced by the two preparations.

The reduction in hypothalamic-pituitary-adrenal (HPA) suppression by the delayed release prednisolone preparation compared with that produced by the regular rapid release formulation has been confirmed in a longer term study (English, Chakraborty & Marks, unpublished observations) and would seem to be clinically advantageous. These data and conclusions are at variance with those reported by D'Arcy *et al.* (1971) and Turner *et al.* (1973). According to D'Arcy *et al.* (1971) little HPA depression was discernible next morning after prednisolone had been given either as a regular or sustained release preparation during the preceding 24 h, at a 7.5 or 15 mg dose level. Turner *et al.* (1973), using a more sophisticated analytical system similar to our own, found no significant reduction in plasma cortisol levels on the day following administration of prednisolone (10 mg) by mouth to ten healthy volunteers.

The markedly larger percentage of administered prednisolone excreted unchanged in the urine following regular prednisolone compared with the sustained release form may relate to the higher peak levels achieved with the former preparation and the possible saturation of the limited capacity of prednisolone (cortisol) binding globulin present in the plasma. In this situation more unbound prednisolone would be present in the plasma, permitting increased filtration at the renal glomeruli. Since prednisolone and cortisol compete for the same high affinity binding globulin in the plasma, it is the sum of their plasma concentrations, rather than that of either alone, which determines the amount of ultrafilterable steriod present in the blood at any one time.

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(Received October 17, 1974)