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DETERMINATION OF PREDNISONE AND PREDNISOLONE IN HUMAN SERUM BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY —ESPECIALLY ON IMPAIRED CONVERSION OF CORTICOSTEROIDS IN PATIENTS WITH CHRONIC LIVER DISEASE

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SUMMARY

A reliable and rapid method is described for the determination of prednisone and prednisolone in human serum by high-performance liquid chromatography, using a Zorbax-SIL column with dichloromethane–ethanol (92.5:7.5) as eluent, with UV detection at 254 nm.

Metabolites and endogenous hydrocortisone did not interfere with the determination of prednisone and prednisolone. The alteration of corticosteroid concentrations in serum from patients with chronic liver diseases was studied following a single oral administration of prednisone or prednisolone (30 mg).

The proposed method showed good separation of several corticosteroids and was time-saving, suitable and reliable for the routine analysis of corticosteroids in human serum.

INTRODUCTION

The application of high-performance liquid chromatography (HPLC) to the determination of human serum concentrations of synthetic corticosteroids has been reported by several researchers[1–4].

Determination of corticosteroids in human serum requires specificity and high sensitivity, two demands that can be satisfied by using suitable phase systems and efficient columns in HPLC. At the same time, chromatographic parameters and extraction methods should be optimized to determine low concentrations (200–500 pg) with reasonable reliability.

HPLC is very effective for the separation of synthetic corticosteroids, either singly or as a group[5–8]. The practical application for the routine determination of serum prednisone and prednisolone has been studied, and this paper describes an HPLC method that is sufficiently sensitive and specific for the determination of serum prednisone and prednisolone in order to study the dynamic metabolism of synthetic corticosteroids in liver diseases.

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EXPERIMENTAL

Materials

Prednisone and prednisolone (Sigma, St. Louis, MO, U.S.A.) were used for the preparation of standard solutions. Δ^4-Pregnene-17α, 20β, 21-triol-3,11-dione (Sigma) was used as internal standard. Solvents used for mobile phase were dichloromethane (Wako, Osaka, Japan) and ethanol (Kanto, Tokyo, Japan).

Chromatographic conditions

A constant-volume HPLC Shimadzu Model LC-1 type with SIL-injector, and Shimadzu Model UVD-2 UV detector (254 nm), were used. The column Zorbax-SIL (25 cm × 4.6 mm I.D.) containing 5-μm spherical silica gel was packed in the stainless-steel tube purchased from Shimadzu Corporation.

The mobile phase was dichloromethane–ethanol (92.5:7.5) at a flow-rate of 1 ml/min.

Reagent solutions

Prednisone and prednisolone, accurately weighed, were dissolved in dichloromethane–ethanol (9:1) in a 100-ml volumetric flask. An aliquot of this solution was diluted with dichloromethane–ethanol (9:1) to produce a final solution of the desired concentration.

A solution of the internal standard, Δ^4-pregnene-17α,20β,21-triol-3,11-dione, was prepared by a similar procedure.

Procedure

The extraction procedure for prednisone and prednisolone from serum is as follows.

To 0.5 ml of serum, 100 μl of internal standard solution (corresponding to 250 ng of Δ^4-pregnene-17α,20β,21-triol-3,11-dione, 0.2 ml of 1.25 *M* sodium hydroxide and 10 ml of dichloromethane were added. The solution was shaken for 10 min in a triturium, and stored at room temperature for 30 min. The upper phase of the extracted solution was aspirated off, and 1 ml of distilled water was added to 10 ml of the lower phase, then stored for 30 min at room temperature. The 10 ml of the lower phase was evaporated to dryness in a rotary evaporator at 40°C. The residue was dissolved in 100 μl of dichloromethane–ethanol (9:1), and 10 μl of this was injected on to the chromatograph using a SGE 10-μl syringe (North Melbourne, Australia).

Subjects

In order to study the dynamic metabolism of synthetic corticosteroids (prednisone and prednisolone) in liver diseases, rapid prednisone or prednisolone tests were performed on five healthy volunteers and seven patients with decompensated liver cirrhosis. Diagnosis of liver cirrhosis was confirmed by needle biopsy under laparoscopic observation. None of the patients had previously been treated with glucocorticoids. None had been taking drugs believed to induce microsomal enzymes.

After preload blood had been sampled, all subjects orally received (on randomised days) 30 mg of prednisone or prednisolone with 120 ml of water. Peripheral

venous blood samples were drawn after 15, 30, 60, 120, 180, 240 and 360 min, under fasting conditions. The serum was stored at −20°C until analysis.

RESULTS AND DISCUSSION

The chromatogram recorded at 254 nm (Fig. 1) showed satisfactory separation of the steroids, and the following retention times were obtained: prednisone, 4 min 40 sec; cortisol (F), 6 min; prednisolone, 7 min; internal standard, 9 min. It is worthy of note that prednisolone was clearly separated from its metabolites, prednisone and endogenous hydrocortisone.

Fig. 2 shows a chromatogram of a serum blank and HPLC pattern after addition of three standard steroids to serum sample. It is evident that no interfering compounds were extracted from serum and that any endogenous substances did not interfere with simultaneous analysis of prednisone and prednisolone.

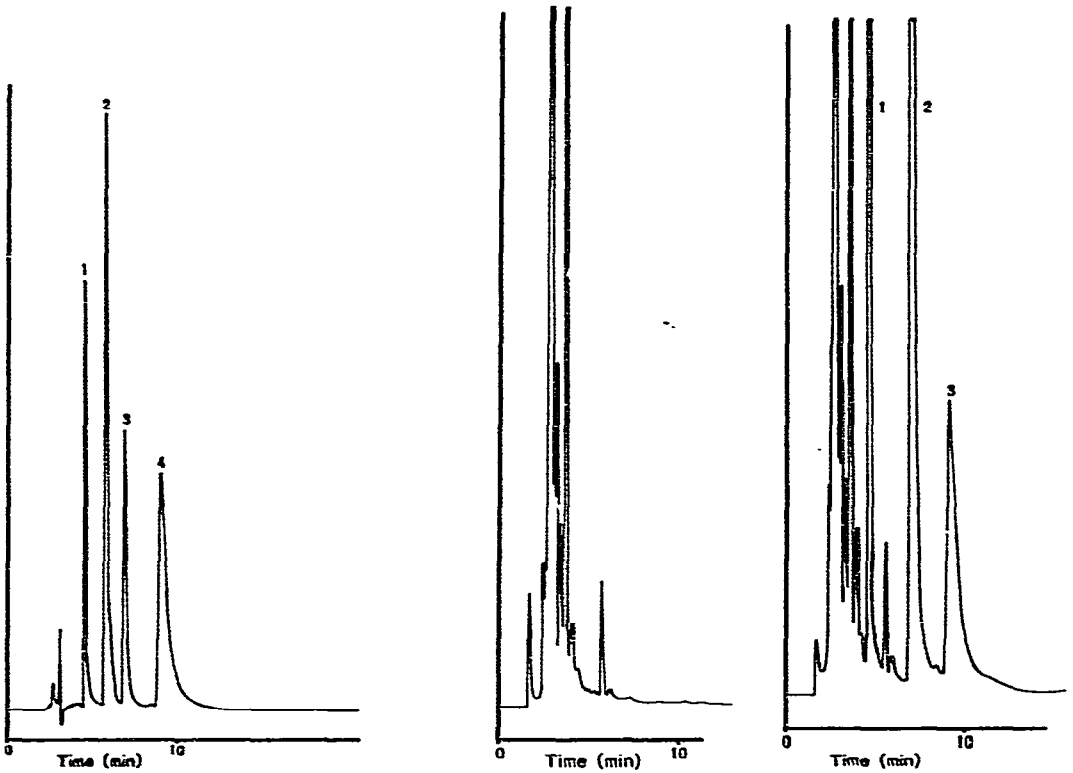


Fig. 1. HPLC profile of t, r standard steroids added to dichloromethane–ethanol (90:10). Peaks: 1 = prednisone; 2 = hydrocortisone; 3 = prednisolone; 4 = Δ^4-pregnene-17α,20β-21-triol-3,11-dione.

Fig. 2. HPLC profile of serum in patient with liver cirrhosis before addition of prednisone (left). HPLC profile of three standard steroids added to serum sample. Peaks: 1 = prednisone; 2 = prednisolone; 3 = internal standard (right).

The calibration curves for the synthetic corticosteroids are showed in Fig. 3.

The coefficients of variation (C.V.), derived from using spiked serum samples were, at the lowest quantifiable limits: prednisone, 1.0%; hydrocortisone, 0.9% prednisolone, 1.2%; internal standard, 1.2%.

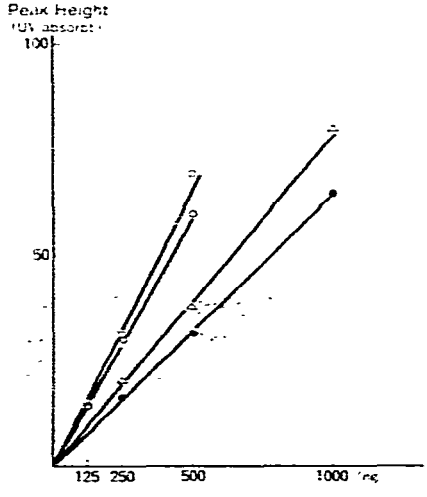
The recoveries of prednisone, prednisolone and internal standard from a pooled serum samples were calculated by chromatographing extracts before and after adding known amounts of these steroids. Constant recovery of 83.4% was observed for each of steroids.

Rapid prednisolone test

A rapid prednisolone test was performed in order to elucidate the reserve function of corticosteroid metabolism in liver disease. Fig. 4 shows the serum concentration of prednisolone after oral administration of prednisolone. There was no significant difference between controls and cirrhosis patients with the respect to the maximal concentrations or to the concentration of prednisolone.

A rapid prednisone test

In the same way, a rapid prednisone test was performed. Fig. 5 shows the HPLC pattern of serum prednisone and prednisolone in patients with liver cirrhosis. The time course of changes in the amount of each of these steroids varied. In the control group, the average maximal concentrations of prednisone and prednisolone were usually identified at 60 min (prednisone, 23.7 ng/ml; prednisolone, 171 ng/ml), then decreased. In the group with chronic liver diseases, the average maximal concentrations of these steroids were identified at 30 min (prednisone, 332 ng/ml; prednisolone, 113 ng/ml), then gradually decreased during 6 h (Fig. 6).



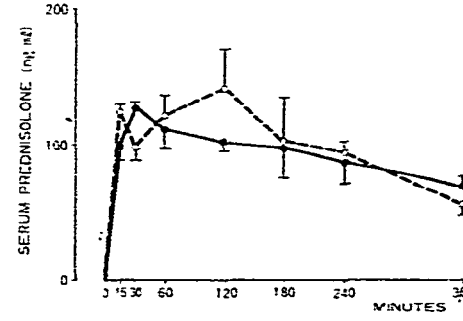


Fig. 3. Calibration curves for the determination of steroids. ●, Hydrocortisone; ○, prednisone; △, prednisolone; □, Δ^4-pregnene-17α,20β,21-triol-3,11-dione.

Fig. 4. Serum concentration of prednisolone after oral administration of prednisolone in patients with liver cirrhosis, and in controls. Mean ± S.E.M. (n = 4). ○ – – – ○, control; ●—●, liver cirrhosis.

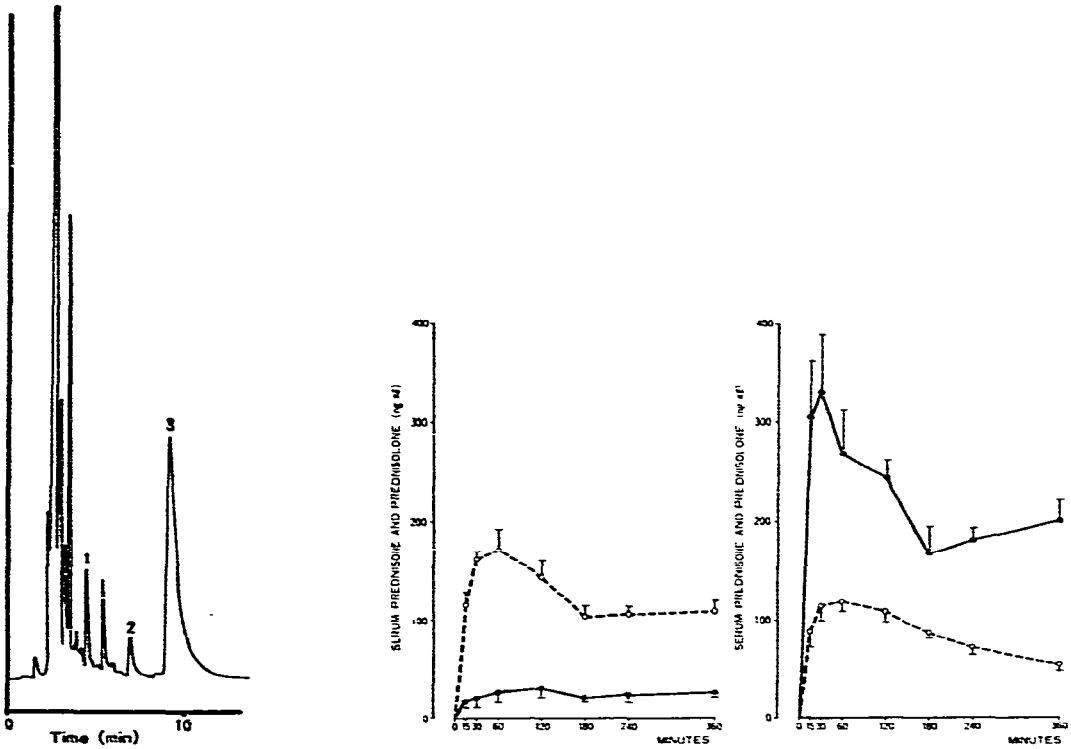


Fig. 5. HPLC profile of serum prednisone and prednisolone in patients with liver cirrhosis given 30 mg of prednisone. Peaks: 1 = prednisone; 2 = prednisolone; 3 = internal standard.

Fig. 6. Serum concentration of prednisone (●— ●) and prednisolone (○ – – – ○) after oral administration of prednisone to patients with liver cirrhosis (right) and controls (left). Mean ± S.E.M.; controls: $n = 5$, patients with liver cirrhosis; $n = 7$.

CONCLUSION

Several methods have been described for the determination of prednisone and prednisolone in human serum[1–4,9]. These methods were sensitive, specific and reproducible; however, long retention times and comparatively complex procedures of extraction effectively prevent their use in a bioavailability trial. The simultaneous determination of prednisone and prednisolone by the present method proved to be sensitive, specific, efficient, reproducible and clinically useful. It has proved robust in use with fast analysis time, less than 12 min for each sample, and no significant problems of late eluting peaks. It is possible to perform more than 20 samples by this method in one day. The procedure described in this paper also facilitates the characterization of prednisone and its pharmacologically active metabolite, prednisolone. The simple procedure described is likely to be available in any laboratory practising HPLC and is cheap and easy to run and maintain.

Prednisone is frequently prescribed for the treatment of chronic active liver disease in western countries, and must be reduced at the 11β-keto group for conversion into its active therapeutic derivative, prednisolone[10]. This conversion depends on an 11β-dehydrogenase, mainly located in the liver[11].

A few studies have been reported of impaired conversion of prednisone into prednisolone in patients with liver cirrhosis[12,13] by means of radioimmunoassay. But this method is not attractive for bioavailability trials, because of cross-reactivity with endogenous cortisone and cortisol[14].

On the basis of the HPLC method described in this paper, it can be concluded that the present method is superior to other techniques for the analysis of corticosteroids, and that simultaneous determination of prednisone and prednisolone by HPLC shows that conversion of prednisone into prednisolone is impaired in patients with liver cirrhosis.

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