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Characterization of prednisone, prednisolone and their metabolites by gas chromatography–mass spectrometry

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ABSTRACT

Human urinary metabolites of the synthetic corticosteroids prednisone and prednisolone were detected in the course of gas chromatographic steroid profiling as methoxime-trimethylsilyl derivatives. Metabolites were provisionaly identified by combined gas chromatography–mass spectrometry. The major metabolites were 11-keto/11-hydroxy conversion products, 20-hydroxy and 4,5-dihydro analogues of the parent drugs. Cortisone, 6-hydroxy and fully saturated A-ring compounds were minor metabolites. Retention indices and mass spectral data are presented.

INTRODUCTION

Synthetic corticosteroids (SC) are extensively used for the treatment of various diseases. Recently, the gas chromatographic–mass spectrometric (GC–MS) analysis of several SC has been reported: dexamethasone [1,2], triamcinolone [2,3], betamethasone [4,5], methylprednisolone [6,7] and fluorometholone [8]. Using methoxime-trimethylsilyl (MO-TMS) derivatives [2–8] a number of metabolites was identified in human urine. Both GC profiling and GC–MS with selected-ion monitoring (SIM) are suitable for the sensitive detection of SC in human biological fluids.

However, prednisone and prednisolone, which are widely used [9], have not been investigated in this way. An attempt was made to use GC–MS for the identification of metabolites and prednisone was found as a major metabolic product of prednisolone [10]. By means of high-performance liquid chromatography (HPLC) and using a series of synthetic reference steroids, 20β-hydroxy [11] and 6β-hydroxy [12] metabolites were identified in human urine. Exogenous cortisone and cortisol were found as prednisone and prednisolone metabolites in swine plasma using HPLC [13,14].

EXPERIMENTAL

Materials

Prednisone and prednisolone (Serva, Heidelberg, Germany) were adminis-

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tered orally (20 mg). β-Glucuronidase/arylsulphatase from *Helix pomatia*, N-trimethylsilylimidazole (TSIM) and methoxyamine chloride were purchased from Serva. The last compound was dissolved in pyridine (puriss. p.a. grade, Fluka, Buchs, Switzerland) to obtain a 4% solution. Organic solvents were distilled before use.

Sample preparation

Urine samples were collected before and for two days after administration. Free steroids were isolated from urine samples (15 ml) adjusted to pH 9 with potassium carbonate by extraction with 20 ml of diethyl ether. Conjugated steroids were isolated similarly after enzymatic hydrolysis: 15 ml of urine were adjusted to pH 4.5 with acetic acid and 2 ml of acetate buffer, then 200 μl of β-glucuronidase were added and the mixture was incubated for 2 h at 57°C. The ether extract was dried with anhydrous sodium sulphate and evaporated to dryness.

MO-TMS derivatives were obtained according to Curvers *et al.* [15]. Methoximation was performed in 50 μl of methoxyamine solution at 80°C for 1 h. Pyridine was removed under vacuum at 80°C, then 30 μl of TSIM were added and silylation was carried out for 1 h at 100°C. Prior to GC–MS analysis the samples were purified according to Leunissen and Thijssen [16], *i.e.*, dissolved in 0.5 ml of dichloromethane and rapidly washed with 0.05 *M* sulphuric acid (0.5 ml) and twice with distilled water (0.5 ml), followed by evaporation to dryness. The dry residue was dissolved in 10 μl of benzene before injection.

Gas chromatography

A Hewlett-Packard (HP) 5730A gas chromatograph was equipped with an HP fused-silica capillary column (25 m × 0.20 mm I.D.; cross-linked methylsilicone, film thickness 0.11 μm) coupled via a custom-made outlet flow splitter to a nitrogen–phosphorus and a flame ionization detector. Make-up gas (helium) was fed to the splitter at a rate of 15 ml/s. The carrier gas was helium at 27 cm/s. The injection port and detector temperatures were maintained at 300°C. The column temperature was programmed from 220°C (2-min delay) to 280°C at 2°C/min, with an inlet splitting ratio of 1:30. Data handling and chromatogram plotting were carried out by means of an HP 3354 B/C laboratory data system.

Gas chromatography–mass spectrometry

An HP Model 5995 quadrupole mass spectrometer with an HP 9825B computer was used. An HP fused-silica capillary column (12.5 m × 0.20 mm I.D.; cross-linked methylsilicone, film thickness 0.33 μm) was coupled to the ion source via open split interface. The carrier gas (helium) linear flow-rate was 25 cm/s. Splitless injection (0.3-min delay) and a splitting ratio of 1:30 were used. The injector and GC–MS interface were maintained at 290°C. The GC column temperature programma was as follows: 180°C (0.5-min delay), ballistic heating to

220°C and a ramp of 5°C/min to 290°C with a final hold for 10 min. Electron-impact mass spectra were acquired at 70 eV, 300 mA and 200°C in the ion source. Scanning was performed from 70 to 800 for 2 s.

RESULTS AND DISCUSSION

GC profiles of human urinary steroids before and after drug administration

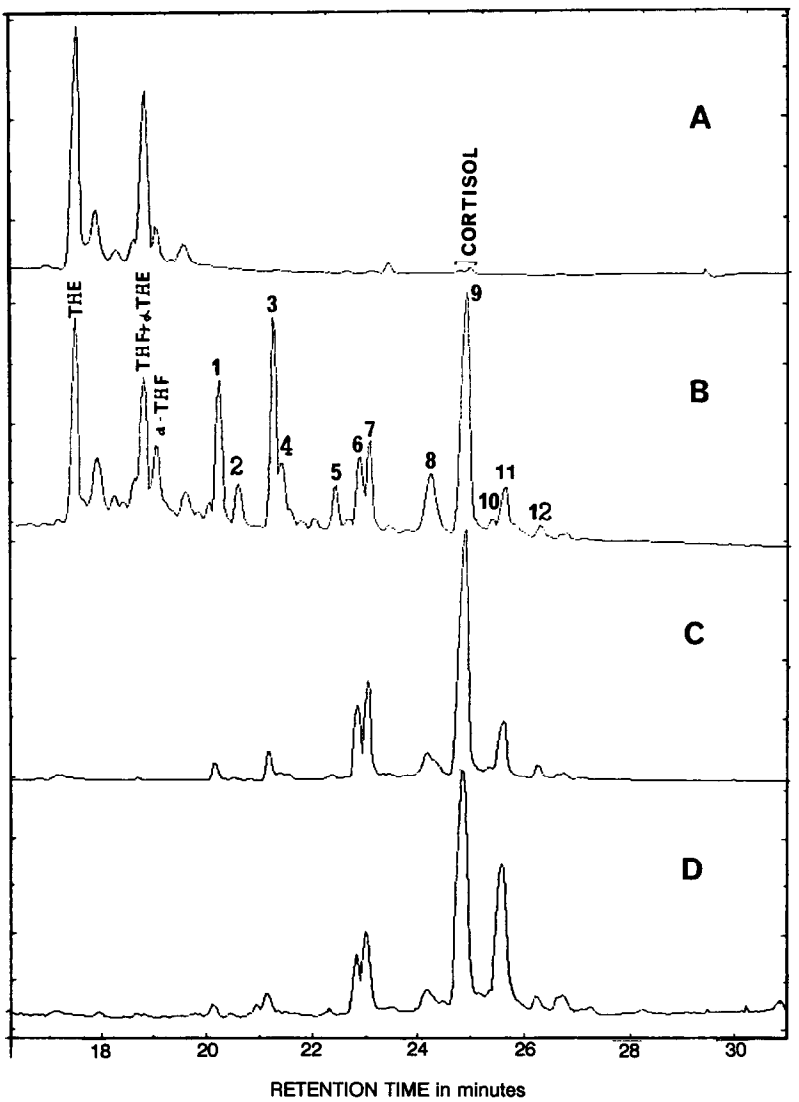

Fig. 1. Sections of GC urine steroid profiles (MO-TMS derivatives): (A) before prednisolone administration (NPD, conjugated fraction); (B) 5 h after administration of prednisolone (NPD, conjugated fraction); (C) 5 h after administration (NPD, free fraction); (D) 5 h after administration (FID, free fraction). THE = tetrahydrocortisone; THF = tetrahydrocortisol.

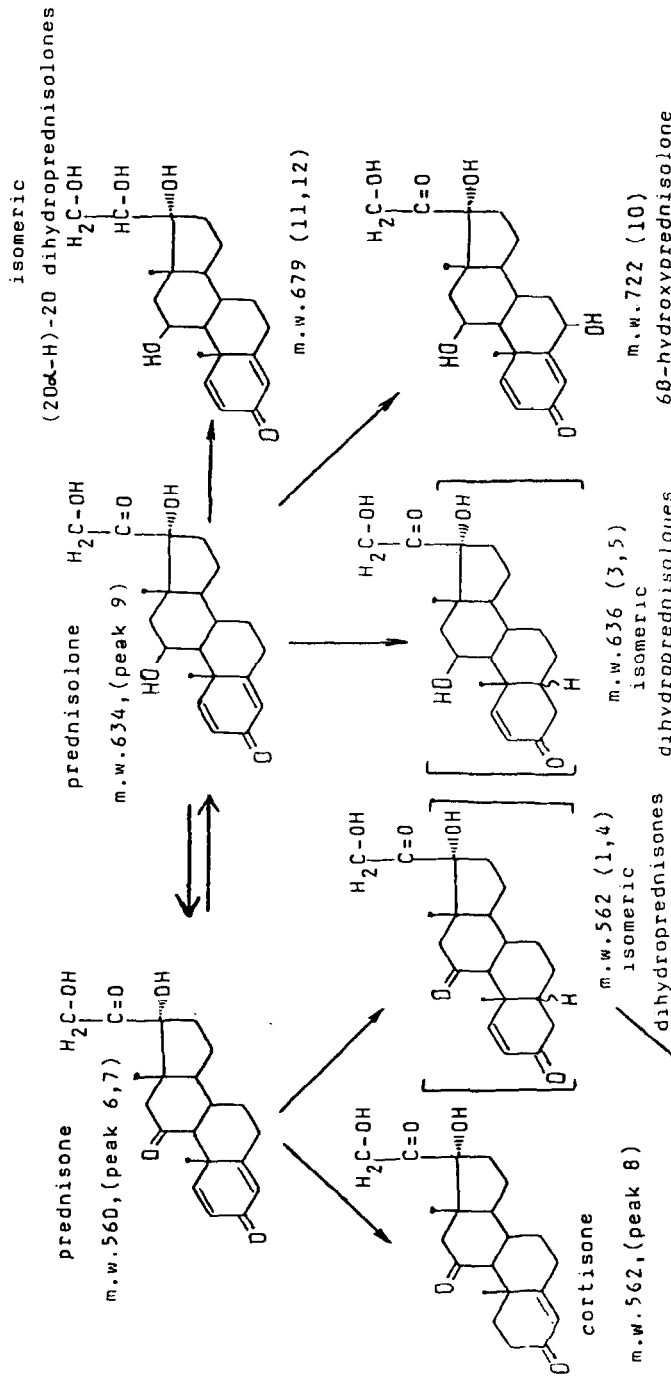

Fig. 2. Proposed prednisone–prednisolone metabolism. The structures of tentatively identified metabolites are given in parentheses.

are presented in Fig. 1. Tetrahydrocortisone (THE), tetrahydrocortisol (THF) with their 5α-isomers and the minor component cortisol were detected in a blank urine (Fig. 1A). Peaks 1–12 appeared after drug administration (Fig. 1B). The retention indices of peaks 6, 7 and 9 correspond to those of authentic prednisone (*syn–anti* isomers) and prednisolone. The others belong to the metabolites formed in the human body. The GC profiles after prednisolone and prednisone administration are identical and independent of which drug was used.

The presence of previously found prednisolone 6β- and 20β-hydroxy metabolites [11,12] was confirmed by MS. The 20β-hydroxy metabolite (peak 11, MW of the derivative = 679) with only one keto group is discriminated on nitrogen–phosphorus detection (NPD) whereas the flame ionization detection (FID) response is significant (Fig. 1C and D). Peak 12 (MW = 679) corresponds to its 20α-hydroxy isomer.

The minor peak 10 represents the 6β-hydroxy metabolite of prednisolone (MW = 722). As shown by Derks and Drayer [17], C-6-hydroxy substitution in the corticosteroid molecule results in a considerable chromatographic separation of the C-3-MO group *syn* and *anti* isomers. The *anti*-C-3-MO isomer of 6β-hydroxyprednisolone coelutes with peak 11, being slightly separated as a shoulder on the right.

TABLE I

GC RETENTION INDICES AND CHARACTERISTIC IONS IN MASS SPECTRA OF MO-TMS DERIVATIVES OF PREDNISONE, PREDNISOLONE AND METABOLITES

Compound	MW	RI	Peak	*m/z* (relative intensity, %, in parentheses)[a]
4,5-Dihydroprednisone metabolite	562	3092 3135	1,4	441(5), 459(10), 531(20)
1,2,4,5-Tetrahydroprednisone metabolite	564	3104	2	377(10), 443(10), 533(28), 564(8)
4,5-Dihydroprednisolone metabolite	636	3130 3174	3,5	246(8), 361(7), 425(8), 515(15), 533(6), 615(25), 636(7)
Prednisone	560	3192 3199	6,7	215(25), 309(30), 399(10), 439(5), 470(5), 529(10), 560(5)
1,2-Dihydroprednisone (cortisone)	562	3248	8	129(25), 147(15), 149(20), 175(20), 262(15), 351(15), 441(15), 472(9), 531(25), 562(6)
Prednisolone	634	3269	9	129(20), 147(20), 149(25), 175(20), 262(25), 293(10), 352(12), 383(8), 391(8), 423(10), 513(15), 544(10), 603(20), 634(6)
6β-Hydroxyprednisolone metabolite	722	3300 3327	10,11	129(10), 219(22), 237(10), 246(6), 484(20), 574(44), 605(20), 632(20), 691(2)
20-Hydroxy metabolite of prednisolone	679	3325 3340	11,12	147(49), 205(95), 378(24), 468(31), 486(28), 499(13), 558(29), 589(16), 648(28), 679(3)

[a] Relative to *m/z* 73 (100%).

A number of dihydro metabolites of prednisone and prednisolone were detected. Following other workers [13,14] we have made an attempt to detect cortisone and cortisol as prednisone–prednisolone 1,2-dihydro metabolites. Only cortisone was found, as peak 8 (MW = 562) as follows from the GC and MS data presented in Table I. Unfortunately, cortisol (Fig. 1A) and prednisolone (peak 9, Fig. 1B) are not separated. Prednisolone, being a major component of peak 9, does not allow cortisol to be detected in the full-scan mode. The *m/z* 634/636 ratio measured in the SIM mode was *ca.* 8, which is close to the theoretical isotope value for pure prednisolone.

For the other dihydro metabolites, 4,5-double bond reduction was assumed. Peaks 1 and 4 (MW = 562) are considered to be isomeric 5α- and 5β-dihydro metabolites of prednisone. The other similar peaks 3 and 5 (MW = 636) are dihydrogenated 5α- and 5β-prednisolone metabolites. The identical peak-area ratios (3:1) in both instances for two considerably separated peaks was observed during excretion. The elution order of 5α- and 5β-isomers was assumed to be similar to endogenous THF and THE, where the β-isomer elutes first [16].

The further increase in molecular weight by 2 u for the peak 2 metabolite (MW = 564) evidently indicates hydrogenation of the remaining double bond in the steroid A-ring. This is a second-stage metabolic product.

The prednisone–prednisolone human metabolites detected and tentatively identified in this study are summarized in Fig. 2. Together with the well known 11-keto–11-hydroxy interconversion and the formation of prednisolone 6β- and 20-hydroxy metabolites, we observed hydrogenation of double bonds in the steroid A-ring. A similar biotransformation was demonstrated by Dumasia and Houghton [18] for the 1,4-diene anabolic steroid 1-dehydrotestosterone. A molecular weight increase by 2 u was noted previously [2,3] for a minor triamcinolone metabolite. Prednisone–prednisolone hydrogenated metabolites are present in significant amounts. One of their interesting features is an ability to form conjugates. Hydrogenated metabolites of the anabolic steroid methandrostenolone [19] also form C-3 conjugates. Conjugate formation is possible after 3-keto group enolization, which may take place in the human body.

A GC profile allows the reliable and sensitive detection of prednisolone–prednisone-positive urine samples, especially when NPD and FID are used. The results may be further confirmed by GC–MS with SIM of characteristic ions, *e.g.*, *m/z* 147, 205, 529, 603 and 648.

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