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Determination of medrogestone in plasma by high-performance liquid chromatography

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Abstract

Interference with the UV absorbance of medrogestone by endogenous steroids in plasma was prevented by reacting plasma with oxalyl chloride. The reduction of interference was effective when oxalyl chloride was in the range 10–50 μl/ml plasma. Reaction of oxalyl chloride with plasma for 10 min could reduce interference approximately 5.5-fold, and there was no significant reduction after 30 min. The limit of quantitative concentration for medrogestone in HPLC was 1 ng/ml. The standard curves were linear with the correlation coefficient greater than 0.999 in the range of 1–30 ng/ml. The coefficients of variation of both intra- and inter-day mean values were <12% and <10% of the actual values, respectively. The developed method for plasma sample preparation and the evaluated HPLC condition were further applied to an in vivo pharmacokinetic study.

Keywords: Medrogestone

1. Introduction

Medrogestone (6,17-dimethylpregna-4,6-diene-3,20-dione) is a synthetic progesteronal agent and is active when orally administrated [1,2]. A protein-binding assay has been developed to determine the serum concentration of medrogestone. This assay, however, is time consuming with low reliability which limits its application [3–6]. Martin et al. [7] have analyzed the concentrations of medroxyprogesterone acetate in biological system by radioimmunoassay, but this nonspecific procedure severely restricts its general applicability to most steroids [8]. The gas chromatographic–mass spectrometric (GC–MS) technique was then developed, and was used to determine the plasma concentrations of medroxyprogesterone acetate [9,10] and megestrol acetate [11] in healthy volunteers after oral administration. Selected-ion monitoring of the molecular ion of medroxyprogesterone acetate trifluoroacetate (m/z 482) allowed quantification of plasma medroxyprogesterone acetate with a lower detection limit of <1 ng per sample. We have prepared a derivative of medrogestone, medrogestone 3-enol trifluoroacetate, for GC–MS analysis [12]. The reproducibility and time taken for the preparation of a derivative for medrogestone is of concern. Robinson et al. [13] developed a high-performance liquid chromatographic method with ultraviolet detection, but limited sensitivity does not allow quantitative determinations in serum volumes of less than 5 ml, and the reported limit of detection is 2 ng/ml. A more specific and more

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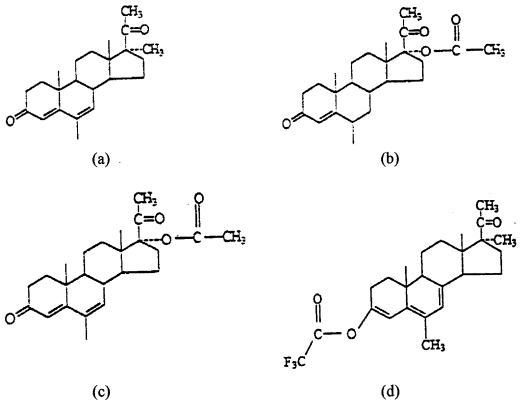

Fig. 1. Chemical structures of (a) medrogestone, (b) medroxyprogesterone acetate, (c) megestrol acetate and (d) medrogestone 3-enol trifluoroacetate.

efficient analytical method was required in order to determine the concentration of medrogestone in biological samples accurately. The chemical structures of medrogestone and its related derivatives are shown in Fig. 1.

The object of this study was to develop and evaluate an HPLC analytical method for the quantitation of medrogestone in plasma. A special treatment for the plasma sample was investigated in order to prevent the UV absorbance of mederogestone from endogenous interference. The developed method for the plasma sample and the validated HPLC analytical method were further applied to a pharmacokinetic study in a group of healthy Chinese male volunteers after oral administration of a 5-mg medrogestone tablet. The related pharmacokinetic parameters were obtained.

2. Experimental

2.1. Materials

Colprone® tablet (Lot. 3cqe-ij, containing 5 mg medrogestone) was obtained from Ayerst Laboratory. Medrogestone standard was purchased from AKZO Pharma Division. Oxalyl chloride was from Wako, Japan.

2.2. HPLC conditions

Medrogestone was assayed by high-performance liquid chromatography (HPLC, Waters) at an UV wavelength of 288 nm (Jasco 875 UV detector). A LiChrosorb Si-60 column (25 cm×4 mm, 5 μm, Merck) eluted with a mobile phase of *n*-hexane–ethyl acetate–tetrahydrofuran (67:23:10, v/v) at a flow-rate of 1 ml/min was employed. Five standard solutions of medrogestone with concentrations ranging from 1 to 30 ng/ml were prepared to construct the calibration curve. The intra- and inter-day precision of HPLC analytical method for medrogestone was validated. Three concentrations representing the lowest, the medium and the highest concentrations of the entire range of the calibration curve, 1, 10 and 30 ng/ml, were studied with six replicates within 1 day, and once daily for 6 days for each concentration. The mean, standard deviation (S.D.) and coefficient of variance (C.V. %) were determined.

2.3. Volunteers

Twelve healthy Chinese male volunteers were included in this study. Their ages were 23.6±2.5 years (range 20–29 years), and their body weights were 64.7±4.9 kg (range 56–75 kg). They passed the physical examination and gave their written consent prior to the study. They were not allowed to take any medication for 2 weeks before or during the study.

2.4. Drug administration and sampling

The 12 volunteers received a single 5-mg dose of medrogestone. They fasted for 12 h (overnight) with subsequent drug administration and then continued to fast for a further 4-h period after dosing. After each drug administration the blood samples were obtained from the forearm vein and collected in heparinized tubes at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 12, 24, 36, 48, 60, 72 and 84 h. The plasma fraction of each blood sample was separated immediately by centrifugation and stored at −20°C in labeled vials until analysis.

2.5. Plasma sample preparation

2.5.1. Effect of amount of oxalyl chloride

One millilitre of blank plasma was deproteinised by 120 μl of ethanol following extracted by 6 ml of isooctane. The supernatant was then removed to a tube containing 40 mg of sodium bicarbonate and various volumes of oxalyl chloride (10, 20, 30, 40 and 50 μl) and allowed to react for 60 min. Two millilitres of 1 *M* sodium borate solution was added into the mixture, and the supernatant was collected and dried. Finally, 250 μl of isooctane was added, and the solution was assayed by HPLC. The peak area under the chromatogram at ~6.8 min was recorded.

2.5.2. Effect of reaction time of oxalyl chloride

The same procedures as described above was conducted, except 20 μl of oxalyl chloride was added and reacted for 10, 20, 30, 45 and 60 min, respectively.

2.5.3. Blood sample

One millilitre of plasma sample was prepared as described above, except 20 μl of oxalyl chloride was added into the extract and reacted for 30 min. If the concentration of medrogestone in blood samples was below 1 ng/ml, a double quantity of plasma and solvents were used and reconstituted with half the volume of isooctane (125 μl) to achieve a concentration of medrogestone within the range of calibration curve. The intra-day and inter-day precision were also validated to assure the reliability of the present procedure.

2.6. Data analysis

All data were first treated by PCNONLIN for curve fitting. The plasma concentrations were analyzed using the open two-compartment model,

$$C(t) = Ae^{-\mathrm{kat}} + Be^{-\alpha t} + Ce^{-\beta t} \quad (1)$$

where k_a is the first-order absorption rate constant, α is the first-order distribution rate constant, β is the first_order elimination rate constant, *A*, *B* and *C* are the corresponding constants for three phases, and $C(t)$ is the plasma drug concentration at time *t*, and the related pharmacokinetic parameters were obtained. Noncompartment model in the computer program, LAGRAN-P, was used to obtain statistical moment parameters AUC_∞ and $AUMC_\infty$. By assuming medrogestone followed linear pharmacokinetics, the Cl_p/F, V_p/F and V_{dss}/F values were calculated as follows:

$$\frac{Cl_p}{F} = \frac{\mathrm{Dose}}{AUC_\infty} \quad (2)$$

$$\frac{V_p}{F} = \frac{\mathrm{Dose}}{C_0} \quad (3)$$

where Cl_p, V_p, V_{dss} and F represent plasma clearance, volume of distribution of central compartment, steady state volume of distribution and bioavailability factor, respectively.

3. Results and discussion

3.1. Plasma sample preparation

Fig. 2a is the HPLC chromatogram for medrogestone with concentration of about 15 ng/ml, and the retention time is ~6.8 min. Fig. 2b is the HPLC chromatogram for blank plasma without oxalyl chloride treatment. The interference by endogenous substances in plasma was observed around the position of medrogestone peak. Deghenghi and Gaudry [14] have reported that the UV absorbance of endogenous steroids could be shifted to a shorter wavelength (~242 nm) after reacting 3-ketone group on the structure with oxalyl chloride. Fig. 2c is the HPLC chromatogram for blank plasma followed by oxalyl chloride treatment. It is obvious that the interference by endogenous steroids in plasma was reduced significantly.

The effect of reduction of endogenous interference by oxalyl chloride was similar when oxalyl chloride was in the range of 10–50 μl (Fig. 3). Reaction of oxalyl chloride with plasma for 10 min could reduce interference by approximately 5.5-fold, and there is no significant reduction after 30 min (Fig. 4). Therefore, 20 μl of oxalyl chloride and reaction time of 30 min were selected for the in vivo plasma

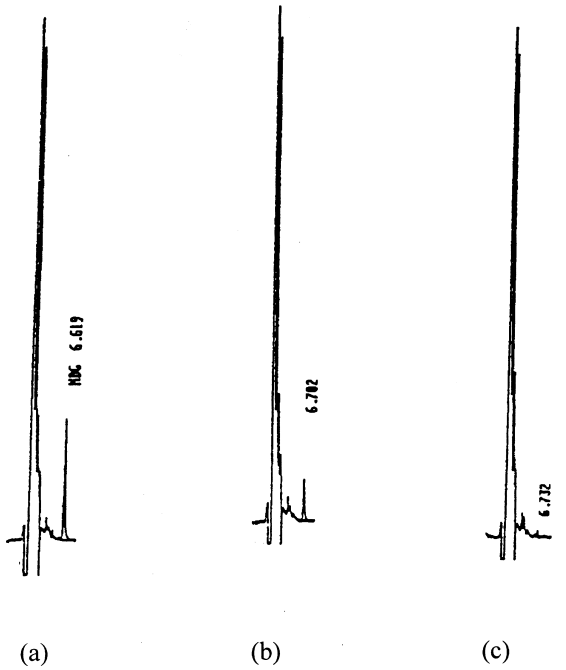

Fig. 2. HPLC chromatograms for (a) medrogestone (15 ng/ml); (b) blank plasma without reacting with oxalyl chloride; (c) blank plasma after reacting with oxalyl chloride.

samples preparation. Since the structure of medrogestone contains a 4,6-diene-3-ketone, the conjugated double bond made medrogestone more difficult to react with oxalyl chloride than endogenous steroids. The recovery of medrogestone from plasma followed by oxalyl chloride treatment was greater than 90%. The production of more volatile by-products, CO and CO_2, during chlorination moved the reaction toward completion even at room temperature (22°C). Sodium bicarbonate was added to neutralize another reaction by-product, HCl, in the solution, and the unreacted oxalyl chloride in the extract was then removed by aqueous alkaline sodium borate.

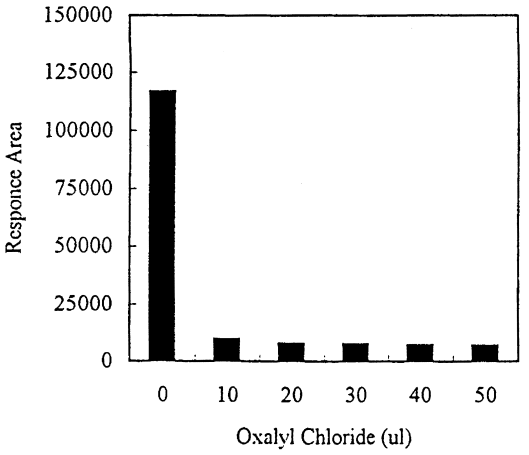

Fig. 3. Relationship between the amount of oxalyl chloride and the response area of blank plasma in HPLC chromatogram at ~6.8 min.

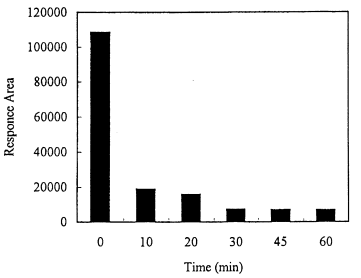

Fig. 4. Relationship between the reaction time of oxalyl chloride and the response area of blank plasma in HPLC chromatogram at around 6.8 min.

3.2. HPLC conditions

The limit of quantitative concentration for medrogestone was 1 ng/ml, and the retention time was 6.8 min. To determine the linearity, the blank plasma was spiked with medrogestone at a concentration range of 1–30 ng/ml, and the standard curves were linear with the correlation coefficient greater than 0.999 over the working range of concentrations. The precision of this analytical method was evaluated, and the coefficients of variance of both intra- and inter-day mean values were $<12\%$ and $<10\%$ of the actual values, respectively (Tables 1 and 2).

3.3. In vivo pharmacokinetic study

The mean medrogestone plasma concentrations of 12 volunteers are shown in Fig. 5. The related pharmacokinetic parameters are listed in Table 3. It is obvious that the inter-individual variation existed after the administration of the same amount of medrogestone. The difference between maximum and minimum values of C_{max} was about four-fold (15.57 and 4.12 ng/ml), and the time to reach the

Table 1
Intra-day precision for medrogestone in plasma

Run no.	Concentration (ng/ml)		
	1	10	30
1	0.91	10.07	29.78
2	1.22	10.07	30.35
3	1.22	10.22	30.00
4	1.33	10.04	29.59
5	1.21	10.70	29.58
6	1.19	10.06	28.78
Mean	1.18	10.36	29.68
S.D.	0.14	0.42	0.53
C.V. (%)	12.08	4.01	1.78

Table 2
Inter-day precision for medrogestone in plasma

Day no.	Concentration (ng/ml)		
	1	10	30
1	1.46	10.09	28.55
2	1.28	9.11	30.06
3	1.46	10.74	29.32
4	1.49	11.75	29.82
5	1.52	10.40	28.82
6	1.28	9.90	30.02
Mean	1.42	10.33	29.43
S.D.	0.11	0.89	0.64
C.V. (%)	7.53	8.57	2.18

Table 3
Pharmacokinetic parameters for medrogestone based on two compartment model ($n=12$)

Parameters	Mean	S.D.	C.V. (%)
C_{max} (ng/ml)	8.21	2.78	33.9
t_{max} (h)	2.57	1.02	39.7
AUC_{∞} (ng h/ml)	98.05	41.46	42.3
AUC_t (ng h/ml)	83.94	42.43	50.5
$AUMC_{\infty}$ (ng h^2/ml)	3485.37	1667.41	47.8
Cl_p/F (l/h)	0.06	0.02	33.3
V_{dss}/F (l)	2.08	1.12	53.8
$t_{1/2}$ (h)	34.95	17.04	48.8

C_{max}, t_{max}, also showed a big difference ranging from 1 to 3.5 h. The significant inter-individual variation was reflected in the extraordinarily large standard deviation.

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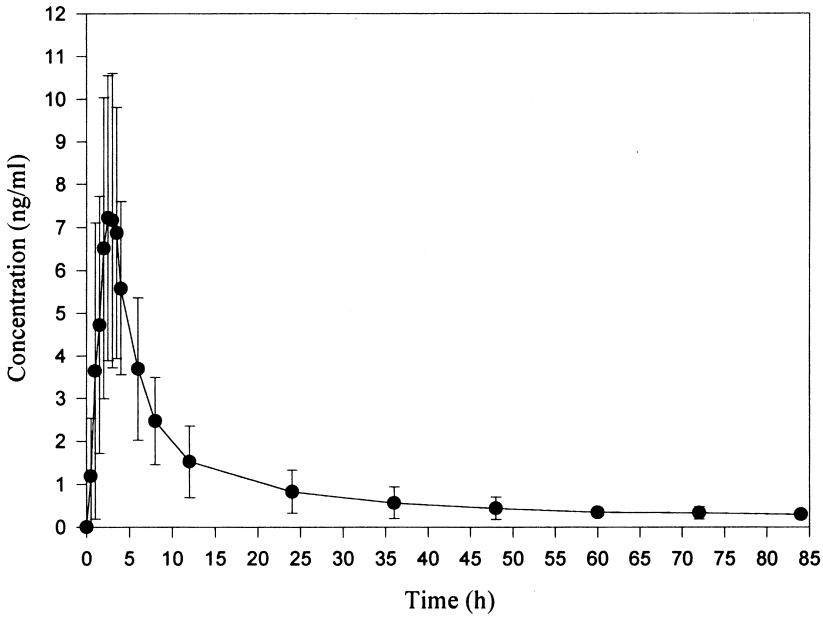

Fig. 5. Mean plasma concentrations of medrogestone in healthy Chinese male volunteers following oral administration of a 5 mg tablet.

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