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Note

Determination of medroxyprogesterone acetate in plasma by high-performance liquid chromatography

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Medroxyprogesterone acetate (MPA) is a synthetic progestational agent which has proved to be effective for contraceptive purposes [1] and is increasingly used in oncology as an adjuvant treatment for hormone-dependent cancers such as endometrial cancer [2] and especially breast cancer [3, 4]. The plasma measurement of this product in breast cancer, for which repeated high doses of MPA are becoming frequent, appears to be relevant, as was recently demonstrated in a comparative study on kinetics during per os or intramuscular administration [5]. Reports in the literature make mention of gas chromatography [6] or radioimmunology [7] for quantification of MPA concentrations in the blood. We propose a method based on high-performance liquid chromatography in which emphasis has been placed on the ease of plasma extraction and on the speed and quality of the chromatographic resolution.

MATERIALS AND METHODS

Reagents and materials

Bidistilled water was obtained from Laboratoire Aguettant (Lyons, France), R.P. Normapur methanol was obtained from Prolabo (Paris, France), and glacial acetic acid was purchased from Carlo Erba (Milan, Italy). Medroxy-

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progesterone acetate was a generous gift from Carlo Erba (Milan, Italy): batch MP 0004/2149. Cyproterone acetate was a generous gift from Laboratoires Theramex (Principality of Monaco). The internal standard, 19-norprogesterone (19-NPG), and the other steroids tested in the study were obtained from Sigma (St. Louis, MO, U.S.A.). Plasma samples were taken into EDTA tubes.

Extraction procedure

Sep-Pak extraction cartridges were purchased from Waters Assoc. (Milford, MA, U.S.A.). Before use they were washed with 2 ml of methanol and 5 ml of distilled water. Use was made of injection syringes with a flow-rate of approximately 2—3 ml/min. A plasma standard enriched with MPA to a concentration of 200 ng/ml was prepared for reference purposes. Plasma samples were tested only once. To 2 ml of plasma (standard or sample) were added 20 μl of 19-NPG $5 \cdot 10^{-5}$ *M* in solution in ethanol. The entire 2 ml were than passed through the cartridge and the eluate was discarded. Five millilitres of water and 2 ml of methanol—water (30:100, v/v) were then passed successively through the cartridge with the eluate being discarded each time. Two millilitres of pure methanol were then passed through the cartridge; the eluate was collected and evaporated at 60°C in a water bath under a flow of nitrogen. The dry residue was recovered in 200 μl of ethanol and an aliquot was injected into the chromatograph. The maximum injected volume necessary to guarantee good separation was 75 μl. We generally used 50 μl.

Apparatus and chromatographic conditions

The liquid chromatograph and column were from Waters Assoc.; the unit included a 6000 A pump and a U6K injector coupled with a radial compression RCM 100 module equipped with a Radial Pak cartridge (8 × 100 mm) containing 5-μm reversed-phase particles of Bondapak C_{18}. An M 440 UV detector (254 nm) connected to a Data Module integrator was used for detection and quantification of the compounds chromatographed. For calculations, peak heights were taken into consideration.

The mobile phase consisted of a methanol—water mixture (75:100, v/v). The pH was lowered to 4.00 with glacial acetic acid. The flow-rate was 2.5 ml/min, and the resulting pressure was 80 bars.

RESULTS

Chromatogram

Fig. 1A shows a chromatogram of blank plasma to which the internal standard 19-NPG (peak 2) had been added; Fig. 1B shows the chromatographic profile of a plasma sample taken 6 h after drug ingestion (1000 mg) and spiked with 19-NPG. MPA was chromatographed and peak 1 corresponds to the retention time of the pure product.

Resolution between MPA and the internal standard 19-NPG appears satisfactory; the blank does not show any interfering peak at the MPA retention time.

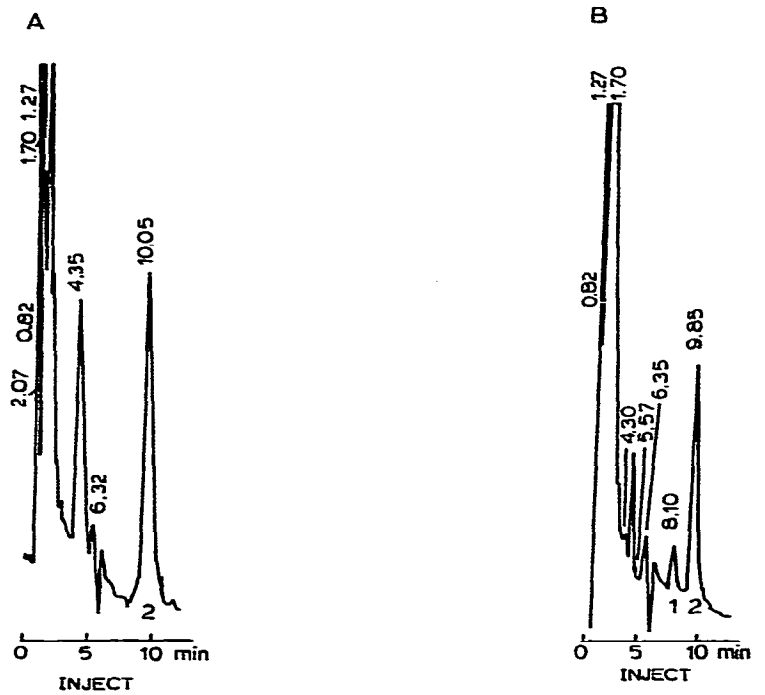

Fig. 1. (A) Chromatogram of a blank plasma sample (2 ml) spiked with 20 μl of $5 \cdot 10^{-5}$ *M* 19-norprogesterone (peak 2); 75 μl injected. (B) Chromatogram of a plasma sample (2 ml) taken 6 h after oral administration of MPA and spiked with 20 μl of $5 \cdot 10^{-5}$ *M* 19-norprogesterone (peak 2); 50 μl injected. Peak 1 corresponds to MPA.

Calibration curve and sensitivity

The calibration curve obtained with MPA concentrations from 20 to 500 ng/ml (six points) was linear, with a coefficient of correlation of 0.987. The limit of sensitivity for plasma samples was reached at a concentration of 4 ng/ml. This represented 2% of full scale detection at 0.005 ABS.

Recovery and reproducibility

Recovery from a reference plasma at a concentration of 200 ng/ml was 76% (the mean of seven samples). Six identical aliquots of plasma (200 ng/ml) were measured in the same series and gave a coefficient of variation [(S.D./mean) × 100] of 3.2%, representing the intra-assay reproducibility.

The same plasma (200 ng/ml) was also evaluated in six different series, and a coefficient of variation of 8.6% was obtained; this represented the inter-assay reproducibility.

Selectivity

The following steroids have been separated using the same chromatographic system: oestradiol, progesterone, promegestone, cortisone, hydroxycortisone, testosterone, dihydrotestosterone and cyproterone acetate. None of them had the same retention times as MPA or 19-NPG.

Application: plasma levels in patients undergoing treatment

MPA was measured for breast cancer patients taking the drug daily, either orally or by intramuscular injection. Table I gives the plasma drug concentrations for the first day and days thereafter. There appear to be wide inter-individual variations in absorption as well as in the steady-state level, as recently mentioned by others [5].

TABLE I

PLASMA LEVELS OF MEDROXYPROGESTERONE ACETATE (MPA) IN BREAST CANCER PATIENTS TREATED ORALLY OR BY INTRAMUSCULAR INJECTION

Means of administration	Patient	MPA concentration in plasma (nmol/l)								
		First day (hours after administration)				Following days (6 h after administration)				
		+2	+4	+6	+12	+1	+2	+3	+4	+5
Intramuscular (500 mg)	1	38	47	22	ND*	5	11	22	28	44
	2	15	30	31	26	30	40	92	27	54
	3	16	54	22	ND	58	50	69	57	53
Oral (1000 mg)	4	118	117	112	12.5	ND	166	170	125	220
	5	11	6	4	<4	14	60	70	75	90
	6	630	530	312	25	120	220	265	147	413

*ND = not determined.

DISCUSSION

The high-performance liquid chromatographic method described allows MPA separation and quantification in plasma. The choice of Sep-Pak cartridges for MPA extraction was based on a previous report [8] concerning estrogen extraction from urine and plasma. The procedure is simple, rapid, quantitative and reproducible, as shown by the relatively low coefficient of variation (below 10%) for intra- and inter-assay reproducibility. It eliminates the need for liquid—liquid extraction, which is often tedious for steroids and can involve classical problems with emulsions and low recovery levels. Radial compression columns represent a new technique in liquid chromatography: the radial compression forms a non-voiding chromatographic bed and applies uniform pressure to the flexible-walled cartridge. The theoretical elimination of channels thus obtained results in objective improvement in separation performance with a greater number of plates when compared to conventionally used stainless steel columns. Furthermore, the back pressure is relatively low: less than 68—100 bar with a flow-rate of 2.5—3.0 ml/min.

We feel that this type of chromatography is particularly suitable for the separation of steroids. All of the major steroids have been tested for possible interference with MPA retention, but none of them showed any superposition of peaks with MPA. It should also be noted that cyproterone acetate, a drug recently shown to have the same retention as MPA [9], did not interfere in this chromatographic system.

While the sensitivity of our method is comparable to that of gas chromato-

graphy [6], our technique has the advantage of being simple and thus easy to perform, especially for sample preparation. Since it allows MPA quantification in patients treated by this drug, it could be used for pharmacokinetics studies. The metabolism of MPA in humans has not yet been studied extensively [10], and our method could be adopted by investigators with a view to identifying and quantifying the products of MPA degradation in man.

REFERENCES

1 P.C. Schwallie and J.R. Assenzo, Fert. Steril., 24 (1973) 331.
2 J. Bonte, J.N. Decoster, L. Ide and G. Billiet, Gynecol. Oncol., 6 (1978) 60.
3 F.M. Muggia, P.A. Cassileth, M. Ocho, Jr., F.A. Flatow, P. Gellhorn and A. Hymang, Ann. Intern. Med., 88 (1968) 328.
4 F. Pannuti, A. Martoni, E.R. Pollutri, P. Camera and G.R. Lenaz, IRCS Med. Sci., (1974) 1605.
5 M. Salimtschik, H.T. Mouritsen, J. Loeber and E. Johansson, Cancer Chemother. Pharmacol., 4 (1980) 267.
6 D.G. Kaiser, R.G. Carlson and K.T. Kirton, J. Pharm. Sci., 63 (1974) 420.
7 M. Hiroi, F.Z. Stanczyk, V. Goebelsmann, P.F. Brenner, M.E. Lumkin and D.R. Mishell, Jr., Steroids, 26 (1975) 373.
8 R. Heikkinen, T. Fotsis and H. Adlercreutz, Clin. Chem., 27 (1981) 1186.
9 G.R. Cannell, R.H. Mortimer and M.J. Thomas, J. Chromatogr., 226 (1981) 492.
10 M. Ishihara, R.Y. Kirdani, Y. Osawa and A.A. Sandberg, J. Steroid Biochem., 7 (1976) 65.