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Plasma concentrations of megestrol acetate and medroxyprogesterone acetate after single oral administration to healthy subjects

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Abstract: Plasma concentrations of megestrol acetate (MA) were measured by radioimmunoassay (RIA) after a single oral dose of 60 mg either in the form of one tablet, or four 15 mg tablets, to 10 women 21–40 years old using a cross-over design. No statistically significant difference between the two preparations was observed with respect to plasma concentrations, the area under the curve from 0 to 24 h or the maximum concentration (c_{max}). For comparison, data are presented on the plasma level of medroxyprogesterone acetate (MPA) following a single oral dose of 100 mg given using a cross-over design in two different tablet forms to 10 healthy men, when no significant difference was observed for these parameters. The mean c_{max} for MA after 2.6 h was 43.9 ng/ml (range 21.7–87.7 ng/ml), whereas that for MPA at 3.1 h was 13.1 ng/ml (range 4.4–29.5 ng/ml) despite the higher dose. After 24 h immunoreactive MA and MPA ranged from 9.6 to 29.0 ng/ml and from 0.2 to 4.0 ng/ml respectively. Moreover, it was found that petroleum ether extraction gives the most specific result by RIA, although considerable amounts of metabolites are still co-estimated. By comparison with selected ion monitoring using GC–MS, metabolite interference in RIA increases with time after administration of the steroids and is considerably greater for MPA than for MA. It is concluded that after oral administration the relative bioavailability of MA is significantly better than that of MPA.

Keywords: *Megestrol acetate; medroxyprogesterone acetate; bioavailability; radioimmunoassay; gas chromatography–mass spectrometry; selected ion monitoring.*

Introduction

Progestins, such as megestrol acetate (MA) and medroxyprogesterone acetate (MPA) have been used for many years in the treatment of advanced endometrial cancer and breast cancer. For endometrial cancer a response rate of 30 to 40% has been reported following MA treatment [1–4]. The response seems to be dependent on the degree of tumour cell differentiation. In breast cancer objective remission after MA therapy has

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been observed in 30% of the patients while stabilization of the condition occurred in 36% [5]. By separating patients into two groups, one estrogen-receptor positive (ER+) and the other estrogen-receptor negative (ER−), it has been observed that more than 50% of the ER+ cases show remission during treatment with MPA or MA [6–9]. Although the significance of the distribution of other receptors, like those for progesterone, androgen or prolactin in cancer tissue is still incompletely understood, the possibility of selecting patients for specific hormone treatment emphasizes the potential importance of measuring individual plasma hormone concentrations. These data would permit treatment dosage to be adjusted accordingly in an effort to increase response rate. Evidence has recently been presented to suggest a relationship between increased response and increased plasma progestin concentration [10]. Although plasma concentrations may not always reflect drug concentration at the receptor sites, they can be readily obtained and reflect the degree of absorption, and the rate and extent of metabolism of the drug.

Whereas several plasma concentration studies after therapeutic doses of MPA have been reported [10–25], relatively little data on MA levels in patients are available [12, 26, 27]. Further information on intra- and inter-subject variations of MA plasma levels would be useful in order to ensure adequate therapy through individual dosage adjustment. Consequently, the present work reports the plasma concentrations of MA after single oral administration to healthy adult female subjects. By way of comparison, some new data on the plasma concentrations of MPA after oral administration to male subjects are also presented, together with relevant results of some methodological studies.

Materials and Methods

Subjects

Ten healthy adult female volunteers, 21–40 years of age, were included in the study. Exclusion criteria were: oral or parenteral medication within the preceding 2 weeks; abnormality of the gastrointestinal tract; history of malabsorption or medical illness attributable to the GI tract; renal or hepatic disease; disposition to thromboembolic disease; pregnancy or lactation. In the experiments with MPA, 10 healthy young and middle-aged male volunteers were involved.

Clinical experiments

Each female subject received either 60 mg of MA orally as tablets (Niagestin® 60), or the equivalent as four 15 mg tablets (Niagestin® 15; Novo Industri A/S, Bagsvaerd, Denmark), together with 120 ml of water at 8 a.m. in the morning. The subjects fasted from 8 p.m. on the day preceding drug administration until 4 h afterwards. Thereafter, the subjects were allowed to eat and drink normally. After one week the experiment was repeated using a cross-over design. Blood samples (5.0 ml) were taken from a forearm vein 0, 0.5, 1.5, 2, 4, 6, 8, 12 and 24 h from the start of each experiment. The blood was collected in heparinized tubes, the plasma separated by centrifugation and stored at −20°C until analysis.

The ten male subjects each received 100 mg of MPA orally in the morning after fasting overnight. Half the subjects were given a 100 mg tablet of Provera® (Upjohn International, Kalamazoo, U.S.A.), while half were given another 100 mg tablet preparation (Orion-Yhtymä, Helsinki, Finland). After a few weeks the experiment was

repeated using a cross-over design. Blood samples (10.0 ml) were drawn into heparinized tubes from a forearm vein 0, 1, 2, 3, 4, 5, 6, 8 and 24 h from the start of the experiment and plasma was stored at −20°C until analysis.

Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC–MS) was carried out using the LKB model 9000 instrument (LKB Instruments AB, Bromma, Sweden), equipped with a column packed with 1% SE-30 and coupled on-line to a Hewlett–Packard HP-2100 A computer (Hewlett–Packard, Palo Alto, CA, U.S.A.) as previously described [28]. For selected ion monitoring (SIM), a four-channel multiple-ion detector (Altema, Stockholm, Sweden) coupled to the GC–MS instrument was used.

The determination of MA by SIM was carried out as previously described [26, 27]: 1 to 2 ml plasma is extracted with ether–chloroform (3:1 v/v), chromatographed on silica gel, the methoxime derivative formed and the ions at *m/z* 310 and 312 monitored, using MPA as internal standard. Originally the same method was used for MPA, but because of the lower concentration of MPA found in plasma, the sensitivity of the method was improved by adopting the following new method: 3 ml of plasma (containing MA as internal standard) is extracted with 2 × 5 ml of petroleum ether, evaporated to dryness and the residue transferred to a 5 × 0.5 cm Lipidex™ 5000 column in two aliquots of 0.2 ml of methanol–water–chloroform (9:1:2 v/v); the MPA fraction and the internal standard is eluted with 2.5 ml of the same solvent. After evaporation, 100 μl of saturated ethoxyamine hydrochloride in pyridine is added and the tubes incubated at +60°C for 60 min [29]. The pyridine is evaporated, 0.5 ml of water added and the mixture is extracted with 3 × 0.5 ml of ethyl acetate. The solvent is evaporated to dryness and the residue dissolved in a suitable amount of ethyl acetate for GC–MS. The ions at *m/z* 324 (the ethoxime of the internal standard, MA) and at 326 (MPA ethoxime) are monitored. This method proved to suffer less interference by cholesterol and is more sensitive due to better behaviour of the ethoxime derivative compared to the methoxime derivative during GC–MS. The practical sensitivity limit for each steroid is between 0.3 and 0.5 ng/ml of plasma. Since both MA and MPA are rather strongly adsorbed in the inlet of the LKB 9000 instrument, sensitivity is lost, so that 24 h after oral administration of 100 mg of MPA the plasma levels are already at the sensitivity limit of the SIM method. The new method is also suitable for the more sensitive analysis of MA, using MPA as internal standard.

Radioimmunoassay of megestrol acetate and medroxyprogesterone acetate

The radioimmunoassay (RIA) methods utilize an anti-MPA-3-(O-carboxymethyl) oxime–bovine serum albumin serum, which cross-reacts to the extent of about 50% with MA, but not with endogenous steroids, as previously described [12, 14]: extraction with petroleum ether is followed by RIA, using radioactive internal standards. The sensitivity limit for both steroids, as calculated from the variation of the blank values in plasma from non-treated subjects, is 0.1–0.2 ng/ml. In the present study the intra-assay precision for MA was found to be 4% (relative standard deviation (RSD) of differences between duplicate assays). In previous studies the intra-assay and inter-assay RSD values for MA were found to be 8.9 and 10.6% at 16 ng/ml respectively [12], while the corresponding values for MPA were 4.7 and 11% at 2–7 ng/ml, respectively [14].

If the same antiserum is used different extraction techniques yield very different results in the RIA assay of both MA and MPA as discussed previously [12, 14]. With

solvents such as benzene–iso-octane (3:1 v/v) [13, 30] or diethyl ether [16, 17, 21, 24, 31], the degree of overestimation is huge ([14] and Table 1). Petroleum ether extraction [11, 12, 14, 22, 23, 32] gives the most specific results (Table 1). The yield of the 'specific' MA or MPA fraction after chromatography on Lipidex 5000, following earlier petroleum ether extraction, is similar to that after ether extraction. However, only 28% (MA) or 30% (MPA) of the immunoreactive metabolites extracted with ether are extracted with petroleum ether, as calculated from results of triplicate experiments with plasma pools II and III (Table 1). The unconjugated metabolites of MA and MPA in plasma are unknown both with regard to structure and biological activity. It has, however, been suggested that the MA metabolites may be 2-hydroxy-derivatives [12] and these can be expected to cross-react with the antiserum.

Table 1
Effect of various published extraction techniques on the amount of immunoreactive metabolites measured by radioimmunoassays for megestrol and medroxyprogesterone acetate. Pooled plasma was obtained from subjects after oral administration of the drugs. The extracts were subjected to chromatography on Lipidex™ 5000 in petroleum ether–chloroform (95:5 v/v) followed by a final fraction of methanol [12]. The immunoreactivity of all fractions, except those containing the reference standards (added to plasma and chromatographed in the same system), was considered to be due to metabolites

Extraction solvent	Pool No.	Megestrol acetate (metabolites as % of total immunoreactivity)	Medroxyprogesterone acetate (metabolites as % of total immunoreactivity)
1 × 10 vol. of benzene–iso-octane (2:1 v/v)	I	—	48
3 × 5 vol. of ether–chloroform (3:1 v/v)	I	—	48
2 × 5 ml of petroleum ether	I	—	13
2 × 5 vol. of diethylether	II	—	69*
2 × 5 vol. of petroleum ether	II	—	28*
2 × 5 vol. of diethylether	III	41*	—
2 × 5 vol. of petroleum ether	III	16*	—
MA and MPA standard added to drug-free plasma and extracted with 2 × 5 ml of petroleum ether		2.6†	4.9†

* Mean of three experiments; plasma pool collected 3 h after administration of drug.
† Demonstrates effect of plasma constituents on the behaviour of the reference standards in the chromatographic system used. This amount of immunoreactivity occurred in the metabolite fractions.

Methodological experiments

After administration of 50 mg of MA orally to four female subjects the plasma levels of MA were measured at specified time intervals using both RIA and SIM [12]. The level of immunoreactive metabolites as a percentage of total immunoreactivity was calculated from the differences of the mean values of the RIA and SIM results (Table 2). Similarly, plasma samples taken at specified intervals from the 10 male subjects given MPA were pooled separately for each of the two MPA tablet preparations, after which both RIA and SIM measurements were carried out. Because the results differed very little for the two drug preparations the mean values were calculated (Table 2).

Table 2
Estimate of the content of immunoreactive metabolites determined by radioimmunoassay of megestrol acetate and medroxyprogesterone acetate after petroleum ether extraction. The results are expressed as the percentage of the total immunoreactivity of the difference between radioimmunoassay results and results obtained by selected ion monitoring

Megestrol acetate		Medroxyprogesterone acetate	
Time after drug administration (h)	Percentage metabolites*	Time after drug administration (h)	Percentage metabolites†
		0.5	21.4
1	26.5	1	53.4
2	31.5	2	66.7
2.5–3	14.8	3	58.9
3.5–4	18.6	4	60.7
5–5.5	21.7	5	61.7
6–7	49.0	6	67.9
7.5–9	42.2	8	73.3
24–25	54.7	24	92.2

* Calculated from data in [12]. Mean of experiments in four subjects.
† Mean of two experiments carried out with pooled plasma from 10 male subjects after administration of 100 mg of two different MPA tablet preparations (Upjohn and Orion; see text).

Results

The administration of a total of 60 mg MA either as a single tablet or as 4 × 15 mg tablets in cross-over design to 10 healthy female volunteers revealed no statistically significant difference for the two preparations with respect to plasma concentrations at each sampling time, the areas under the curve (AUC_{0-24}) and the peak concentrations. Further analysis on the pooled data material for the two preparations was carried out in order to estimate the intra- and inter-subject variations. The plasma concentration with regard to time over the period 0–24 h from two single administrations of 60 mg MA are shown in Fig. 1 as the mean plasma concentrations of the 10 subjects at each sampling time. Maximum values (mean 43.9 ng/ml, range 21.7–87.7 ng/ml) occur after 1–4 h, as shown in Table 3. After 24 h immunoreactive MA ranged from 9.6 to 29.0 ng/ml. There

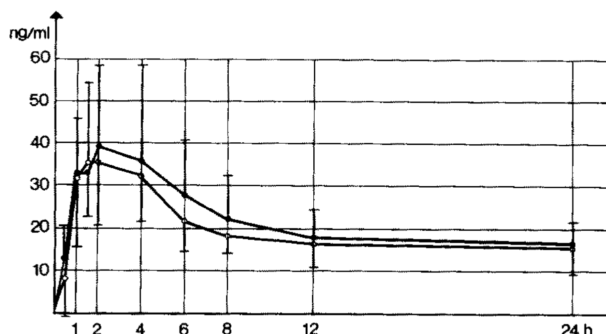

Figure 1
Mean plasma concentrations (with SD) of megestrol acetate after oral administration of a single dose of Niagestin® 60 mg (open circles) and a single dose of four tablets of Niagestin® 15 mg (closed circles) to 10 healthy adult women using a cross-over design, with an interval of 7 days between doses.

Table 3
Peak concentrations (c_{max}), times of peak concentrations (t_{max} and areas under the curve 0–24 h (AUC_{0-24}), for each subject following administration of 60 mg of megestrol acetate (one 60 mg tablet or 4 × 15 mg tablets in a cross-over design) on two occasions (I and II) with an interval of seven days

Subjects	c_{max} (ng/ml)		t_{max} (h)		AUC_{0-24} ((ng/ml).h)	
	I	II	I	II	I	II
1	87.7	52.1	4	1.5	967	731
2	45.5	33.6	2	4	353	384
3	48.5	67.3	2	1.5	449	544
4	28.1	61.0	1	1.5	397	619
5	21.7	30.1	4	4	288	513
6	42.1	42.9	4	1.5	475	542
7	37.7	27.3	1	4	517	467
8	22.0	66.1	1	4	321	678
9	35.2	22.0	4	4	401	350
10	48.9	58.4	1	1	392	511
Mean	41.7	46.1	2.4	2.7	456	534
Grand Mean	43.9		2.6		495	

were still detectable concentrations (range 0.7–3.2 ng/ml), probably representing immunoreactive metabolites, when the drug was administered the second time 7 days after the first experiment.

For each subject the AUC_{0-24} was estimated using the trapezoidal rule (Table 3). For the parameters c_{max} and AUC_{0-24} the two observations for each subject made possible an analysis of variance to estimate both inter- and intra-subject variation. The total variation in c_{max} was composed of two almost equal parts: the estimated standard deviation due to inter-subject variation was 17.9 ng/ml, whereas the standard deviation due to intra-subject variation was 17.6 ng/ml. The overall mean c_{max} was 43.9 ng/ml, representing a standard deviation of about 40%. A similar pattern was found for AUC_{0-24}. The estimated inter-subject standard deviation was 176 (ng/ml).h and the intra-subject standard deviation was 161 (ng/ml).h. The overall grand mean AUC_{0-24} was 495 (ng/ml).h, so that the two calculated standard deviations represent approximately 35% of the mean.

The administration of two different tablet preparations of 100 mg MPA in a cross-over design to a group of 10 male subjects revealed no statistically significant difference for the two preparations with respect to plasma concentrations at each sampling time, the areas under the curve and the peak concentrations (Table 4). The plasma concentration with regard to time over the period 0–24 h after oral administration of a single dose of 100 mg of Provera to the 10 male subjects, after oral administration of a single dose of 100 mg of MPA from Orion to the same 10 male subjects and of a single dose of Niagestin 60 mg to 10 women are shown in Fig. 2 as the mean plasma concentrations of the groups of 10 subjects at each sampling time. Maximum values after administration of MPA (mean 13.1 ng/ml, range 4.4–29.5 ng/ml) occur after 2–5 h (Table 4). After 24 h about 2 ng/ml (range 0.2–4.0) of immunoreactive MPA was

Table 4
Peak concentrations (c_{max}), times of peak concentrations (t_{max}) and areas under the curve 0–24 h (AUC_{0-24}), for each subject following administration of 100 mg of Provera® (A) or 100 mg of another MPA preparation (Orion-Yhtymä B), in a cross-over design on two occasions with an interval of several weeks

Subjects	c_{max} (ng/ml)		t_{max} (h)		AUC_{0-24} ((ng/ml).h)	
	A	B	A	B	A	B
1	5.6	22.0	5	3	54	121
2	8.0	7.6	3	3	102	93
3	19.1	11.7	3	3	185	53
4	29.5	5.3	2	3	150	41
5	12.7	4.4	3	3	95	19
6	9.8	12.5	5	3	171	142
7	9.6	12.2	3	3	65	114
8	11.3	26.0	3	3	80	195
9	8.9	16.6	3	3	80	123
10	19.5	8.6	2	3	136	83
Mean	13.4	12.7	3.2	3	112	98
Grand mean	13.1		3.1		105	

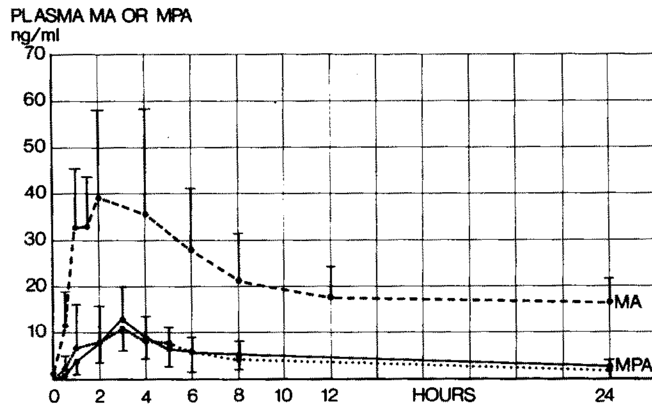

Figure 2
Mean plasma concentrations (with SD) of megestrol acetate (MA) after oral administration of a single dose of Niagestin® 60 mg to 10 healthy adult women, and of medroxyprogesterone acetate (MPA) after administration of a single dose of Provera® 100 mg (Upjohn) (dotted line) and of a single 100 mg tablet (Orion-Yhtymä) (solid line) to 10 healthy adult men.

detectable. The estimated standard deviation for the two preparations due to inter-subject variation of c_{max} was 54%, while for AUC_{0-24} it was 47%. Comparison of the plasma concentrations after MPA administration with those found after administration of MA reveal that, despite the higher dose, MPA concentrations are much lower than those of MA.

Discussion

The data in Tables 1 and 2, together with earlier data [12, 14], clearly indicate that extraction even with a non-polar solvent such as petroleum ether leads to an overestimate of both MA and especially MPA by RIA. This overestimate increases with time after drug administration. Data in the literature based on using the same type of antibody indicate that MPA levels obtained by direct methods without extraction [33] are an order of magnitude higher than values after petroleum ether extraction [11, 12, 32]. Extraction with benzene–iso-octane [30] or diethyl ether [16, 17, 21, 24, 31] also leads to gross overestimation both of MA and MPA (Table 1). The present work supports the view that one chromatographic step is not sufficient to make the RIA assay specific for MA and especially not for MPA. However, combination of the RIA method with extraction and two chromatographic steps, or of the SIM method with extraction and one chromatographic step, is too complicated and expensive for routine clinical work. It is suggested that only petroleum ether be used for extraction, for optimum specificity for both MA and MPA, otherwise the results reflect the metabolite concentrations rather than the parent compounds.

In a previous study on four subjects given 50 mg MA orally, the mean t_{max} was 3.1 h [12, 27] compared to a mean t_{max} of 2.6 h in the present study. In the present investigation assays were carried out at 2 and 4 h, but not at 3 h, which may account for this difference. The present study confirms earlier work, where up to 4-fold differences of c_{max} between subjects were found. The mean c_{max} in the earlier study was 56.9 ng/ml measured by RIA and 44.0 ng/ml assayed by SIM after administration of 50 mg of MA orally [12, 27]. However, in the present investigation, after 60 mg of MA orally it was lower (43.9 ng/ml).

The large differences in the plasma concentrations for MA and MPA, although the difference in structure is only minor (one double bond more between C_6–C_7 in MA), seemed to be of great theoretical interest and perhaps of practical significance. The data are not strictly comparable, since the test subjects were of different sex and the dose was different. However, the difference is so large (Fig. 2), that it is unlikely to be due solely to experimental differences.

The limited data available using SIM [12] indicate that the elimination of the steroids comprises one fast and one slow component. The half-life ($t_{1/2}$) between 3 and 8 h after administration of MA or MPA is about 2.2–2.5 h for both steroids, but after 8 h $t_{1/2}$ is about 14 h for MA and 7 h for MPA. From Table 2 it can also be observed that the relative amounts of metabolites measured using the same RIA method is much higher for MPA; this may be an indication of faster (and/or different) metabolism for MPA. In earlier studies the $t_{1/2}$ of radioactive MPA after a single injection was 230 min [34]; however, these measurements also included the conjugated fraction. According to another study the biological $t_{1/2}$ of MPA was found to be 14.5 h [35], but in this study the urinary metabolites were measured. These values are therefore not comparable with the present data based on a specific GC–MS assay. However, it can be concluded that the elimination of MA seems to be slower than that of MPA. Moreover, it is possible that both compounds, especially MPA, are poorly absorbed. To achieve the same c_{max} of original compound in plasma after oral administration, about ten times more MPA than MA would be needed, if the data in Tables 2, 3 and 4 are taken into account. On this basis, the relative bioavailability of MPA would seem to be much less than that of MA.

It has previously been shown that MA is very resistant to metabolism in various

biological systems compared to MPA [36, 37], so that MA may escape transformation by intestinal bacteria and by enzymes in the intestinal mucosa and in the liver. Another factor which may significantly influence the level of unconjugated MPA in plasma is that MPA is subject to conjugation as such before metabolism occurs, mainly with glucuronic acid [38]. Whether MA can form similar conjugates is uncertain, but it is less likely that such conjugates would occur, because the second double bond in ring B close to the C_6-methyl group may inhibit enolization at C_3. Thus it seems reasonable to suggest that extensive metabolism of MPA may be an important factor involved in the considerably lower plasma concentrations relative to those of MA.

The inter-subject variation of c_{max} and AUC_{0-24} was greater for MPA than for MA, as judged from the RIA results. Because of the extensive use of MA and especially of MPA for the treatment of various hormone-dependent cancers, this large inter-subject variation indicates a strong need for plasma level monitoring during treatment. The present work shows that the methodological problems are great, but that since specificity and plasma levels are relatively high after the dosage commonly used for cancer treatment, the use of MA would make monitoring of plasma concentrations more reliable. However, no studies on the correlation between the effects of these drugs on cancer and their true plasma concentration have yet been carried out.

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