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Comparison of Mass Spectrometry and Radioimmunoassay to Measure Medroxyprogesterone Acetate in Patients with Endometrial Cancer

Jorma Kärkkäinen, Ervo Vesterinen, Ulf-Håkan Stenman, Herman Adlercreutz, Usko Nieminen and Olof Widholm

Serum medroxyprogesterone acetate (MPA) was measured by radioimmunoassay (RIA) and gas chromatography-mass spectrometry (GC-MS) in patients with endometrial cancer. Samples were obtained 3, 6 and 24 h after the oral administration of 100 or 200 mg MPA once a day. The levels obtained by GC-MS were lower (median 16–29%) than those obtained by RIA, which is probably attributable to the presence of metabolites interfering with the RIA. Two commercial MPA formulations gave different MPA serum levels by both RIA and GC-MS. The levels obtained by GC-MS were so low that frequently only partial saturation of the endometrial progesterone receptor may be achieved which may explain why high oral doses are needed to produce optimum therapeutic response.
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INTRODUCTION

THE DAILY doses of medroxyprogesterone acetate (MPA) in breast and endometrial carcinoma range from 100–200 mg to several grams per day. Significant dose–response effects have been reported even between such high doses as 500 and 1500 mg per day [1]. However, in many other well-controlled studies no differences have been found between various high-dose regimens. It is therefore possible that above a certain threshold differences in drug concentration are unimportant.

The commonest method for measurement of MPA in serum is radioimmunoassay (RIA). High serum levels have been measured by RIA after oral and parenteral administration in volunteers [2, 3] and in patients undergoing high-dose treatment [4, 5]. However, since none of the available antisera are completely specific for MPA, the results are influenced by MPA metabolites. The gas chromatography (GC) methods give lower plasma levels than RIA and the differences can be remarkable [6]. Extraction with non-polar solvents such as hexane or petroleum ether [2–5, 7] has been used to reduce interference in RIA.

Wide inter-individual variation in serum MPA levels occurs with RIA [2–5]; similar results were obtained with high-pressure liquid chromatography (HPLC) [9, 10] and GC [6, 11–13], which could both be expected to be more specific than RIA. Different commercial MPA formulations can produce different serum levels according to RIA [4] and HPLC [10].

GC-mass spectrometry (GC-MS) in the selective ion monitoring (SIM) mode is generally regarded as the most specific method for measuring serum levels of hormones and drugs. MS methods for MPA and megestrol accetate have been reported [3, 14–18], but are little used clinically. Serum levels obtained by GC-MS and RIA have been compared in two studies. In one report [3] GC-MS gave MPA levels 8–87% of those obtained by RIA after petroleum ether extraction [3]. However, in another study [16], only small differences were seen. In both of these studies the same type of antiserum was used.

We have studied serum MPA levels by both RIA and GC-MS in patients with endometrial cancer during continuous oral therapy with two commercial MPA formulations at two dosages.

PATIENTS AND METHODS

Two series of patients with stage I–II endometrial cancer were investigated. All patients had undergone hysterectomy or

Correspondence to J. Kärkkäinen.
J. Kärkkäinen, E. Vesterinen, U.-H. Stenman, U. Nieminen and O. Widholm are at the Departments of Obstetrics and Gynecology, Helsinki University Central Hospital, and H. Adlercreutz is at the Department of Clinical Chemistry, University of Helsinki, Meilahti Hospital, Helsinki, Finland.

Table 1. Serum levels of MPA measured by GC-MS and RIA in patients with endometrial cancer during continuous oral administration 3, 6 and 24 h after drug intake

	GC-MS (nmol/l)	RIA (nmol/l)	GC-MS/RIA ratio
100 mg MPA (n=13)			
3 h	13 (4.1–35)*	52 (21–127)	0.24 (0.13–0.89)
6 h	9.1 (3.1–25)	25 (11–90)	0.29 (0.15–0.64)
24 h	3.9 (1.0–11)	16 (6.6–25)	0.28 (0.12–0.56)
200 mg MPA (n=5)			
3 h	44 (23–90)	286 (118–564)	0.16 (0.08–0.30)
6 h	47 (17–56)	242 (56–576)	0.19 (0.08–0.30)
24 h	18 (2.3–29)	82 (24–137)	0.16 (0.10–0.43)

*Median (range).

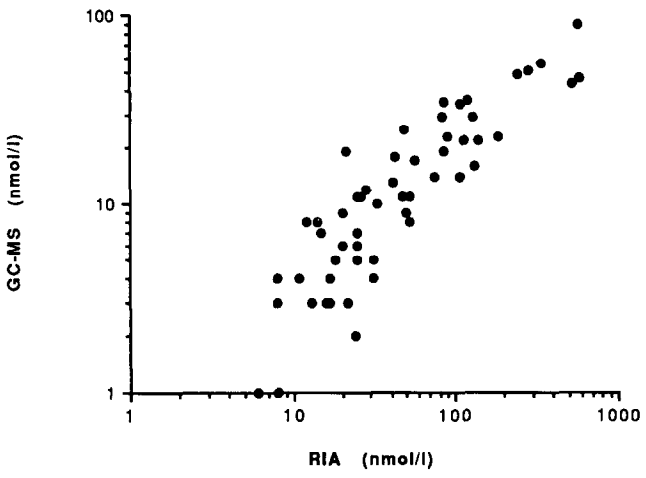

Fig. 1. Serum levels of MPA measured by GC-MS and RIA in patients with endometrial cancer.

bilateral oophorectomy. In both series MPA had been administered for at least a month before the beginning of study. The drug was given as a single oral dose 100 or 200 mg in the morning.

In the first series (n=18, mean age 66.2 [S.D. 8.1] years), blood samples were taken 3, 6 and 24 h after administration of the daily dose of 100 mg (n=7) "Lutopolar" (Medipolar) or 100 mg (n=6) or 200 mg (n=5) "Provera" (Upjohn).

In the second series (n=84, mean age 62.5 [10.5] years) the blood samples were taken 2–5 h after the daily dose of 100 mg (n=28) or 200 mg (n=21) Lutopolar or of 100 mg (n=14) or 200 mg (n=21) Provera. The groups were formed from 133 patients by selecting matched pairs to eliminate bias due to variations of time of blood sampling. In the 100 mg dosage group, 2 patients taking Lutopolar were chosen to match each patient taking Provera.

MPA was assayed by RIA [4, 7]. Petroleum ether was used for extraction. The antiserum was an anti-MPA-3-(O-carboxymethyl)-oxime bovine serum albumin (from goat 16) donated by Upjohn. In the first series GC-MS in SIM mode with megestrol acetate as internal standard was used [3].

RESULTS

The serum levels of MPA in patients in the first series are shown in Table 1. The levels obtained by GC-MS were lower in all samples. The correlation between the results obtained by the two methods is shown in Fig. 1. There was a wide inter-individual variation in the MPA serum levels by both methods. The largest differences between the two methods (lowest GC-MS/RIA ratio) were seen in patients with the highest MPA serum concentrations (P=0.03, 0–24 h, area under the curve [AUC_{24}]) n=18, Spearman rank correlation).

The serum levels obtained after Lutopolar were lower than those after Provera, by both RIA and GC-MS (Table 2), but the differences were not statistically significant (maximum values or AUC_{24}). Table 2 also shows a comparison of the serum levels of MPA (determined by RIA) between the two drug formulations in a larger group (n=84) of endometrial cancer patients in samples taken 2–5 h after the oral administration of the drug. MPA levels after Lutopolar were significantly lower, both in the 100 mg (U test) and in the 200 mg dosage group, than after Provera.

DISCUSSION

The serum levels of MPA by GC-MS method were low compared with RIA in patients with endometrial cancer after oral administration of the drug. The results agree with a previous study [3] in male volunteers but contrast with those obtained in a study of patients with breast cancer [16]. However, either the RIA or the GC-MS method could not have been satisfactory in that study, because the values obtained by the two methods often differed several fold in either direction.

Since the specificity of GC-MS is higher than that of RIA, the results by GC-MS should be regarded as being closer to the true value. Similar results have been obtained for megestrol acetate [3, 7].

A plausible explanation for the discrepancy between the two methods is the formation of metabolites of MPA, especially glucuronides reduced in the A-ring [8, 18, 19]. The use of non-polar solvents (petroleum ether in our study) for extraction considerably reduces the influence of the metabolites. Our study

Table 2. Serum levels of MPA after Lutopolar or Provera

		Lutopolar: MPA (nmol/l)	Provera: MPA (nmol/l)	Mean ratio (Lutopolar/Provera)
Series 1 (100 mg)				
No.		7	6	
3 h	RIA	41 (21–107)	85.5 (25–127)	0.59
	MS	11 (4.1–34)	16.5 (6.2–35)	0.74
6 h	RIA	22 (12–33)	48.5 (11–90)	0.46
	MS	5.2 (3.1–12)	11.5 (3.6–25)	0.47
24 h	RIA	15 (8.2–22)	16.5 (6.6–25)	0.82
	MS	2.6 (1.0–6.7)	5.45 (1.3–11)	0.63
Series 2 100 mg				
No.		28	14	
RIA		16 (2.8–101)*	39.5 (5.0–81)*	0.52
200 mg				
No.		21	21	
RIA		33 (13–177)†	82 (37–255)†	0.48

Lutopolar vs. Provera, two-tailed U test: *P = 0.005 and †P = <0.001.

confirmed earlier studies in that interference in the RIA, even with non-polar solvents, is still so great that the value of the method is questionable for clinical work. Although some of the metabolites may have hormonal activity, this has not been shown and therefore cannot be relied upon.

In our study the difference between the two methods was greatest in patients with the highest plasma levels, as measured by RIA, and greater in samples taken 3 and 6 h after the daily dose than in samples taken immediately before the next dose. This is contrary to that observed after giving single doses of 100 mg to healthy men [3].

There was a significant difference between the serum MPA levels with the two commercial MPA formulations in samples analyzed by RIA. Similar results have been reported with various MPA preparations by RIA [2, 15], HPLC [10] or an enzymatic method [15] but not when two formulations were compared by GC-MS [3]. With RIA the observed differences might be due to the different rates of formation of metabolites and not to the absorption of MPA itself. According to our study, however, this is unlikely because of a difference of similar magnitude between the two drug preparations was also observed by GC-MS and by RIA, although the number of patients studied was too small to make the results statistically significant. The reason why no differences were found between the two MPA formulations in the MS study of Adlercreutz *et al.* [3] is most probably that the two formulations investigated, which were different from those in our work, are equally well absorbed.

Our GC-MS results suggest that the plasma levels of MPA after oral doses of 100–200 mg per day were so low that frequently only partial saturation of the progesterone receptor will occur. MPA binding to albumin [20, 21] leaves only 3.5–5.3% unbound. The equilibrium dissociation constant of MPA for the progestin receptor (0.33 nmol/l) is of the same order as that of progesterone [22]. Consequently when total MPA plasma concentrations are below 5–10 nmol/l, less than 50% of the progestin receptor is saturated with MPA. Complete saturation of the progesterone receptor may not be necessary for optimum clinical response in cancer. However, if the response is dependent on the degree of receptor occupation, the clinical results with oral MPA may be unpredictable.

We found that increases in the oral MPA dose resulted in a greater increase in plasma levels of MPA metabolites than that of MPA itself. It may, indeed, be necessary to use daily doses of several grams, as suggested by Pannuti *et al.* [13] to reach a level of MPA sufficient to saturate receptors. Oral administration of megestrol acetate might be more advantageous [3]. However, no significant clinical differences have been obtained with the two drugs.

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