Cancer Chemother Pharmacol (1990) 27: 101–105

Cancer Chemotherapy and Pharmacology

Influence of aminoglutethimide on the metabolism of medroxyprogesterone acetate and megestrol acetate in postmenopausal patients with advanced breast cancer

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Received 1 November 1989/Accepted 5 June 1990

Summary. In this study the influence of aminoglutethimide (AG) on the disposition of medroxyprogesterone acetate (MPA) and megestrol acetate (MA) was studied. When 1,000 mg AG daily was supplementally given to six patients on chronic treatment with MPA (1,000 mg/day) or MA (160 mg/day), mean serum levels of progestin were reduced by 74% as compared with control levels ($P < 0.03$). AG did not change the blood clearance rate of MPA when the latter was given i.v. This discrepancy between AG's influence on oral and parenteral progestin disposition could be explained by pharmacokinetic properties of the progestins, and our results suggest that AG stimulates the metabolism of progestins. The decrease in MPA and MA serum levels was accompanied by an increase in serum cortisol, sex hormone-binding globulin (SHBG) and testosterone levels. This suggests that AG reduces the biological activity of progestins.

Introduction

Hormonal treatment is the most important form of systemic therapy for advanced breast cancer. Used in postmenopausal women, progestins (medroxyprogesterone acetate, MPA, and megestrol acetate, MA) and aminoglutethimide (AG) produce response rates similar to those obtained with tamoxifen (TAM) [2, 10, 12, 13, 16, 22, 24]. Progestins and AG have different mechanisms of action [11, 12, 21].

Although combined treatment with AG and progestins has been used, no improvements in results have thus far been reported [8, 23]. One study found that patients receiving combined treatment with AG and MPA had lower serum MPA levels than those who were given MPA as a single agent [20]. AG is known to enhance the metabolism of several drugs and steroids that are metabolized by mixed-function oxidases [11]. Although the metabolism of i.v. MPA was found to be unchanged by AG treatment [5], a discrepancy between AG's influence on MPA given orally and parenterally could be explained by pharmacokinetic properties of MPA. MPA is a so-called highly extractable drug [11]. Alterations in the metabolism of such compounds have little influence on their total serum clearance rate; however, they can significantly enchance first-pass metabolism and reduce the serum level when the drug is given by the oral route [11].

Any drug interaction reducing the serum level of MPA or MA may be important for the efficacy of a combined drug schedule. Therefore, this study was initiated to explore further the influence of AG on the pharmacokinetics of MPA and MA.

Patients and methods

Patient characteristics and study design. Six postmenopausal women with advanced breast cancer were included in the study; all gave informed verbal concent to participate. Their mean age was 62.8 years (range, 50–77 years) and their mean body weight was 73.5 kg (range, 48–87 kg). No change in body weight was observed during the study period. Four patients had received previous treatment with TAM, two patients had previously undergone treatment with other progestins (MPA or MA) and three patients had received chemotherapy. Patient 5 was treated with TAM given sequentially with MPA. Two of the women had been oophorectomized; the others were spontaneously postmenopausal (Table 1). None of the patients were smokers. Drugs known to be hepatic enzyme inducers or inhibitors were not given. Other drug schedules (e.g. analgetics) were kept constant during this study.

All of the patients were given hormonal treatment (drug schedule 1) for several months (patient 5 was on MPA for 8 weeks). Each patient was to receive alternative hormone treatment (drug schedule 3) because of progressive disease or relapse. The test measurements were done before the completion of drug schedule 1, after 3 weeks of combination treatment (drug schedule 2) and after 3 weeks of drug schedule 3 (Table 1).

Drug schedules. MA and MPA were given at doses of 160 mg daily and 500 mg b.i.d., respectively, and AG was given at 250 mg q.i.d. with glucocorticoids (hydrocortisone; 50 mg b.i.d. during the initial 2 weeks

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Table 1. Patient characteristics and study design

Patient number	Age (years)	Body wt (kg)	Previous therapy	[^{3}H]-MPA clearance measured	Drug schedule (steady state)		
					1	2	3
1	51	87	T	No	MA	MA+AG	AG
2	67	73	T,CMFP	No	MPA	MPA+AG	AG
3	61	80	C,O,P,CMF,T,L,MPA	No	AG	AG+MA	MA
4	71	48	T	Yes	MA	MA+AG	AG
5	50	83	O,CMFP	Yes	MPA[a]	MPA+AG	AG
6	77	70	MA	Yes	AG	AG+MPA	MPA

AG, aminoglutethimide; C, cyclophosphamide; F, 5-fluorouracil; L, chlorambucil (Leukeran); M, methotrexate; MA, megestrol acetate; MPA, medroxyprogesterone acetate; O, oophorectomy; P, prednisone; T, tamoxifen

[a] T/MPA = T and MPA in sequence (8 week cycles)

and 25 mg b.i.d. thereafter). The same doses were applied to single-drug and combination schedules, with the exception for hydrocortisone, which was omitted when a progestin was given in combination with AG.

Measurements of progestin serum levels. Steady-state serum levels of MA and MPA were measured as follows. All patients received their progestin dose at fixed hours (MA, daily at 8 a.m., MPA, b.i.d. at 8 a.m. and 8 p.m.) for the last 3 days before sampling. After subjects had fasted overnight, blood samples were obtained at 8 a.m. (just before drug administration) and at 2, 4, 6, 8 and 12 h (for MA, also 24 h) after drug ingestion. Blood samples were allowed to coagulate for 1 h and serum was separated by centrifugation and stored at –20°C until analysis. All samples from each patient were analysed in the same run.

Progestin serum levels were determined by RIA after hexane extraction as described elsewhere by the present authors [19]. In serum samples from patients 4–6, both total (unconjugated + conjugated) MPA/MA (direct method, without hexane extraction) and unconjugated MPA/MA serum levels were measured; in the remaining patients, only unconjugated MPA/MA serum levels were assayed.

[^{3}H]-MPA injection study. Patients 4–6 were given [^{3}H]-MPA i.v. in two test situations: (1) while they were on treatment with a progestin only, and (2) while receiving progestin + AG treatment (Table 1). Each injection was given as a 30-s bolus of [^{3}H]-MPA (100 μCi) dissolved in 10 ml 0.9% saline. Each investigation was started at 8 a.m. after patiens had undergone an overnight fast. Blood samples (10 ml) were drawn before the injection and at 3, 5, 7, 10, 15, 30 and 45 min as well as 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 h thereafter. Serum was prepared and stored as described above. Total serum radioactivity and radioactivity in the ether extract (unconjugated MPA) and the residual water phase (conjugates) were measured as previously described [19].

Hormone measurements. With commercially available RIA kits, serum testosterone (T), cortisol and sex hormone-binding globulin (SHBG) were measured in blood samples obtained at 8 a.m. (cortisol, also at 8 p.m.). The intra- and interassay CVs for these RIA assays were between 5% and 10% in our laboratory. Low cross-reactivity is reported for testosterone (ICN Biomedicals, Inc.) and cortisol (Farmos Group Ltd.). No human serum protein is known to cross-react in the SHBG assay (Farmos Group Ltd.)

Pharmacokinetic calculations. Steady-state levels of progestins are given as the mean of the different serum levels measured in a dosing interval. The metabolic clearance rates (MCR) and the distribution volume (V_o) of unconjugated MPA were estimated using the formulas given by Gupta et al. [5]:

$$MCR = \frac{1}{\sum_i A_i/b_i}$$

and

$$V_o = \frac{1}{\sum_i A_i},$$

where A and b are coefficients determined from the functions describing the disappearance curves of MPA using a computer program that applies the Marquarant algorithm [5].

Statistical methods. Statistical comparisons were done by the Wilcoxon matched-pair sign test; all *P*-values are given as two-tailed calculations.

Table 2. Progestin and hormone levels in 6 postmenopausal women with advanced breast cancer during treatment with progestin alone, progestin combined with AG, and AG alone

Tests	During MPA/MA therapy	During MPA/MA+AG therapy	During AG therapy[a]
MPA (ng/ml)[b]	70 ± 31.2	26 ± 9.5	
MA (ng/ml)[b]	177.7 ± 68.1	38.3 ± 27.7	
Testosterone (nmol/l)	1.91 ± 2.33	5.55 ± 4.1	4.13 ± 1.21[c]
Cortisol:			
8 a.m. (nmol/l)	121.8 ±148	390 ±191	361.7 ±159.7[c]
8 p.m. (nmol/l)	34.8 ± 34.2	154 ±156.5	458.7 ±119.3[c]
SHGB (nmol/l)	18.7 ± 15.5	45.9 ± 34.8	56.4 ± 21.4[c]

[a] With hydrocortisone

[b] 3 patients

[c] 4 patients

Values represent the mean ± SD. AG, aminoglutethimide; MPA, medroxyprogesterone acetate; MA, megestrol acetate

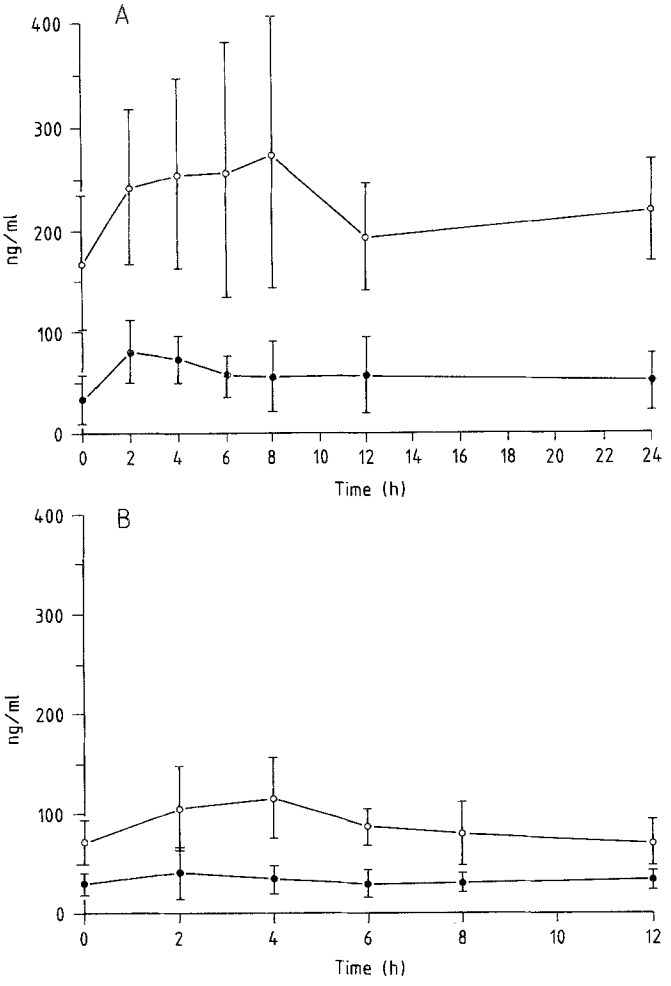

Fig. 1 A, B. A Steady-state serum levels (ng/ml) of MA given alone (□) and in combination with AG (◆). 0 = time at which progestins are given. **B** Steady-state serum levels (ng/ml) of MPA given alone (□) and in combination with AG (◆). 0 = time at which progestins are given

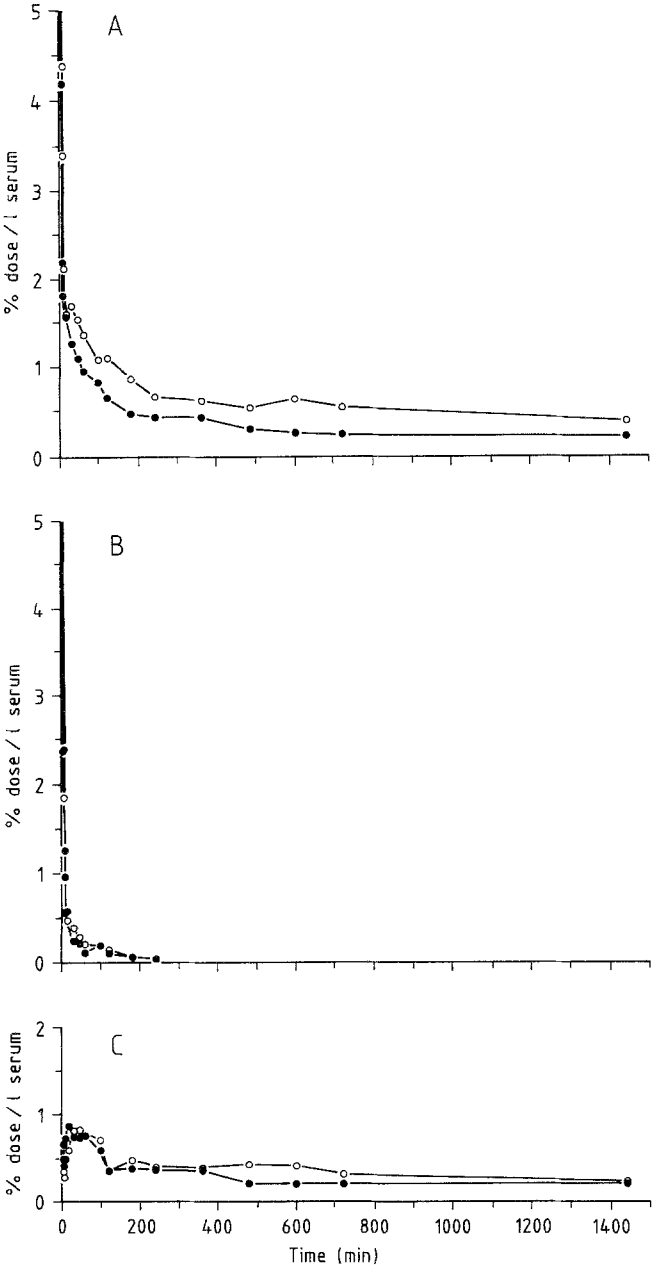

Fig. 2 A–C. Radioactivity expressed (one representative patient) as a percentage of the total dose per litre serum and plotted as a function of time after i. v. injection of 100 μCi [^{3}H]-MPA, during therapy with progestin (□) and progestin + AG (◆). Radioactivity was measured in **A** untreated serum, **B** an ether extract of serum, and **C** the residual water phase

Results

Steady-state serum levels in a dosing interval for MPA (12 h) and MA (24 h), with and without AG, are shown in Fig. A, B. Mean serum levels of MPA and MA at steady state are shown in Table 2. In all patients investigated, AG caused a decrease in serum progestin levels (mean decrease, 74%; range, 27%–86%); this effect was most pronounced for MA (mean decrease, 79% vs 60% for MPA).

Serum levels of MPA ($n = 2$) and MA ($n = 1$) as measured by RIA without hexane extraction (direct assay) resulted in progestin serum values 6–14 times those found after hexane extraction, probably due to high levels of conjugated progestins in serum measured by the direct method [19]. Using this method, a substantial fall in serum progestin values (serum MA or MPA with their corresponding glucuronides) was observed after the addition of AG, for a mean reduction of 60% (results not shown). Serum concentration curves of total radioactivity (unconjugated + conjugated MPA) and radioactivity in the ether phase (unconjugated MPA) and the water phase (conjugated MPA) after i. v. injection of [^{3}H]-MPA in one patient are shown in Fig. 2 A–C; they indicate only a minor effect for the AG combination. Minor effects on the calculated MCR of [^{3}H]-MPA were seen: 1,252 ± 696 l/day during progestin treatment and 1,271 ± 920 l/day during progestin + AG therapy. A large individual difference in V_0 was observed: 8.5 ± 8.1 l during progestin treatment and 1.6 ± 1.5 l during progestin + AG therapy.

The mean serum levels of T, SHBG and cortisol when progestin was given alone were 1.9, 18.7 and 122/35 nmol/l (8 a.m./8 p.m.), respectively (Table 2); during progestin + AG therapy, these values increased significantly, by 186% ($P < 0.05$), 59% ($P < 0.02$) and 221% ($P < 0.02$), respectively.

Discussion

Steady-state serum levels of MPA and MA found in the present study were similar to those previously reported by us [12] and other investigators [20] in patients on similar drug schedules. Interestingly, serum levels of MA were 2- to 3-fold those of MPA (Table 2), although the MPA dose given was 6-fold that of MA. This large discrepancy could be due to an extensive deactivation of MPA in the intestine [14] or to a lower absorption rate of MPA vs MA rather than to differences in first-pass metabolism of the two progestins, as combination therapy with AG caused a greater decrease in serum MA levels than in MPA levels (Fig. 1 A, B).

If MPA is measured in serum by a direct assay (without extraction), mean values of 470 ng/ml [19] and a median of 1,500 ng/ml [1] are found, whereas MPA levels measured in *n*-hexane fractions are about 100 ng/ml [19]; similar results have been reported by other investigators [18]. The antiserum used in our study does not react with MPA or MA metabolites that are modified in the C-or D-ring [18]. The difference in MPA values measured by the direct assay vs indirect method (*n*-hexane extraction) can be explained by the high levels of MPA serum glucuronides that cross-react with the antibodies. This can be avoided if unconjugated MPA is separated from the glucuronides by hexane extraction. We found that cross-reacting materials in the residual water phase after hexane extraction could explain $^2/_3$ of the overestimation found by the direct assay. The serum levels of unconjugated MA and MPA (measured after *n*-hexane extraction) found in the present study are consistent with results obtained by specific methods such as HPLC, gas chromatography-mass spectrometry (GC-MS) and GC [14, 15, 17].

Serum levels of MA and MPA that are necessary for an optimal response are not known. One study suggested a higher response rate in patients with serum MPA levels of >100–150 ng/ml [9], but other investigators could not find any "threshold" [6, 12]. One randomized study of low (300 mg/day) vs high-dose (1,000 mg/day) oral MPA treatment failed to show any difference in response rate [4].

Serum levels of MPA and MA decreased consistently when AG was added. A similar finding was reported by Van Deijk et al. [20] for MPA, but no such study has been conducted using MA. This decrease in MPA and MA serum values could be caused by increased metabolism of the progestins. MA and MPA are known to be metabolized by hydroxylation in different positions of the steroid molecule [3, 7, 14], and hepatic mixed-function oxidases are known to be stimulated by AG [11].

On the other hand, we could not show any influence by AG treatment on the clearance of MPA given parentally as [^{3}H]-MPA; this is consistent with results obtained by Gupta et al. [5]. Several explanations are possible. One is that AG could reduce the uptake of progestins, but there is no evidence to support such a conclusion. Another possibility is that AG induces the metabolism of progestins, but this does not cause a higher serum clearance rate for MPA because MPA clearance approaches hepatic plasma flow [11]. Using the relationship [25]

$$CL = \dot{Q}E = \frac{(\dot{Q} \times CL_i)}{(\dot{Q} + CL_i)},$$

where CL is the hepatic clearance of total drug, $\dot{Q}$ is liver blood flow, E is the hepatic extraction ratio and CL_i is the "intrinsic" clearance rate (the maximal ability of the liver to remove steroid irreversibly from all pathways in the absence of flow limitations), we can see that if intrinsic clearance is high, total clearance approaches the blood flow of the organ as an upper limit. Any change in intrinsic clearance may therefore have little impact on the total clearance rate (CL). The serum progestin values obtained after oral ingestion indicate that enhanced enzyme activity may reduce the amount of drug escaping first-pass metabolism from 10% to 5%, causing a 50% reduction in plasma levels.

Adding AG to MA or MPA treatment caused a significant increase in the mean serum levels of cortisol and T. This finding is most probably secondary to reductions in serum MA and MPA levels with escape from the blocking effect of progestins on the pituitary-adrenal axis. Similarly, the increase in serum SHBG levels caused by AG may be secondary to a reduction in the influence of progestin on hepatic SHBG synthesis. A possible direct effect of AG cannot be exluded due to the lack of untreated control measurements. No cross-reaction with the assay can explain the extremely high levels of serum T found in these women (Table 2).

From our study, a definitive conclusion cannot be drawn as to which metabolic pathways for progestins might be stimulated by AG. The antiserum used in the progestin RIAs cross-react with MPA metabolites modified in positions on the A- and B-rings, but metabolites modified in the C- and D-ring positions do not cross-react [18]. If AG stimulates the hydroxylation of progestin in the A- or B-ring position, such metabolites could be expected to interfere in either the "*n*-hexane" or "total progestin" RIAs. The finding that AG treatment reduced "*n*-hexane" and "total progestin" levels to about the same extent may suggest that AG stimulates progestin metabolism in the C- or D-ring positions. However, to verify such a conclusion, specific antibodies against specific metabolites should be used.

Although clinical studies have thus far shown no benefit for the addition of AG to MPA or MA [8, 23], this could be due to an AG-induced reduction in progestin serum values, which causes a sub-optimal drug level in some patients. Drug schedules combining progestins with a more specific aromatase inhibitor could prove to be of interest.

Acknowledgements. This study was supported by grants from the Norwegian Cancer Society. The authors wish to thank D. Ekse and B. Watne for their excellent technical assistance. Antiserum against MPA was a gift from the Upjohn Co. (Kalamazoo, Mich.).

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